OMB Number: 4040-0001
Expiration Date: 10/31/2019

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

5. APPLICANT INFORMATION **Organizational DUNS*:** 0636907050000

Legal Name*: UNIVERSITY OF ALABAMA AT BIRMINGHAM
Department:
Division:
Street1*: UNIVERSITY OF ALABAMA AT BIRMINGHAM
Street2: 1720 2nd Ave South, (b) (3) (A), (b) (6)
City*: BIRMINGHAM
County:
State*: AL: Alabama
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 352940111

Person to be contacted on matters involving this application

Prefix:	First Name*:	Middle Name:	Last Name*:	Suffix:
Mr.	(b) (3) (A), (b) (6)		(b) (3) (A) (b)	

Position/Title:
Street1*: 1720 2nd Avenue South, (b) (3) (A), (b) (6)
Street2:
City*: Birmingham
County:
State*: AL: Alabama
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 352940111
Phone Number*: (b) (3) (A), (b) (6) Fax Number: (b) (3) (A), (b) (6) Email: (b) (3) (A), (b) (6)

7. TYPE OF APPLICANT* H: Public/State Controlled Institution of Higher Education

Other (Specify):
Small Business Organization Type ❍ Women Owned ❍ Socially and Economically Disadvantaged

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Project 3 - Flavivirus - UTMB

12. PROPOSED PROJECT

Start Date*	Ending Date*
03/01/2019	02/29/2024

OMB Number: 4040-0010
Expiration Date: 10/31/2019

Project/Performance Site Location(s)

Project/Performance Site Primary Location

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	UNIVERSITY OF ALABAMA AT BIRMINGHAM
Duns Number:	0636907050000
Street1*:	UNIVERSITY OF ALABAMA AT BIRMINGHAM
Street2:	1720 2nd Ave South, (b) (3) (A), (b) (6)
City*:	BIRMINGHAM
County:	
State*:	AL: Alabama
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	352940111
Project/Performance Site Congressional District*:	AL-007

Project/Performance Site Location 1

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	The University of Texas Medical Branch at Galvston
DUNS Number:	8007711490000
Street1*:	301 University Blvd
Street2:	
City*:	Galveston
County:	Galveston
State*:	TX: Texas
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	77555-0156
Project/Performance Site Congressional District*:	

Funding Opportunity Number: RFA-AI-17-042. Received Date: 2018-03-29T16:30:43.000-04:00

Project/Performance Site Location 2

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: Oregon Health and Science University
DUNS Number: 0969975150000
Street1*: 3181 SW Sam Jackson Park Rd
Street2: Mail Code L106-OPAM
City*: Portland
County: Multnomah
State*: OR: Oregon
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 97239-3098
Project/Performance Site Congressional District*:

Project/Performance Site Location 3

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: Washington University
DUNS Number: 0685522070000
Street1*: CB 1054
Street2: 1 Brookings Drive
City*: St.Louis
County: St.Louis
State*: MO: Missouri
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 631304862
Project/Performance Site Congressional District*:

Additional Location(s) File Name:

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ○ **Yes** ● **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ○ Yes ○ No

If YES, check appropriate exemption number: _ 1 _ 2 _ 3 _ 4 _ 5 _ 6 _ 7 _ 8

If NO, is the IRB review Pending? ○ **Yes** ○ **No**

IRB Approval Date:

Human Subject Assurance Number

2. Are Vertebrate Animals Used?* ● **Yes** ○ **No**

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ○ Yes ○ No

IACUC Approval Date:

Animal Welfare Assurance Number

3. Is proprietary/privileged information included in the application?* ● **Yes** ○ **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ○ **Yes** ● **No**

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ○ Yes ○ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ○ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ○ **Yes** ● **No**

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	Project_abstract_Mar_14_2018.pdf
8. Project Narrative*	
9. Bibliography & References Cited	Flavi_Bibliography_Final.pdf
10.Facilities & Other Resources	Facilities_Flavivirus.pdf
11.Equipment	
12. Other Attachments	Product_Development_Strategy.pdf

Summary

Many flaviviruses are significant human pathogens, among which dengue virus is the most important mosquito-borne viral pathogen with over 390 million infections per year. Apart from dengue virus, West Nile virus, yellow fever virus, Japanese encephalitis virus, tick-borne encephalitis virus, and recently Zika virus cause global outbreaks and epidemics, posing constant threats to public health. No FDA-approved antiviral drug is currently available to treat any flavivirus infection, and vaccines are lacking against several family members. Thus, the development of flavivirus antiviral therapy is a public health priority. **The goal of this proposal is to develop direct antiviral agents (DAAs) for flavivirus therapy**. We will pursue three Specific Aims to develop flavivirus DAAs. Aim 1. Develop preclinical DAAs targeting flavivirus capsid and NS4B protein. Aim 2. Identify novel Nuc DAAs with pan-flavivirus activity. Aim 3. Evaluate combination therapy directed against flavivirus infection. We will deliver the proposed milestones by combining the cutting-edge biology from academia and drug discovery acumen from industry with clear go/no-go criteria.

FACILITIES & OTHER RESOURCES:

Environment-Contribution to Success: The University of Texas Medical Branch (UTMB) is committed to excellence in research, with several top NIH-funded departments, and is committed to maintaining a strong focus in local, national, and international Health. (b) (3) (A), (b) (4), (b) (6) UTMB contains a fully equipped state-of-the-art, NIH-funded BSL4 facility as well as BSL3 laboratories. The scientific environment is of high quality with Infectious Disease, Bioterrorism and Immunology journal clubs meeting weekly as well as internal and external speakers in relevant seminar series organized by the Departments of Biochemistry and Molecular Biology, Pathology, Microbiology and Immunology, and Medicine and supplemented by the Center for Biodefense and Emerging Infectious Diseases, the Sealy Institute for Vaccine Science, and the Institute for Human Infections and Immunity. UTMB has all of the facilities and major equipment required to make this proposal successful as detailed below.

Facilities:

(b) (3) (A), (b) (4), (b) (6)

(b) (3) (A), (b) (4), (b) (6)

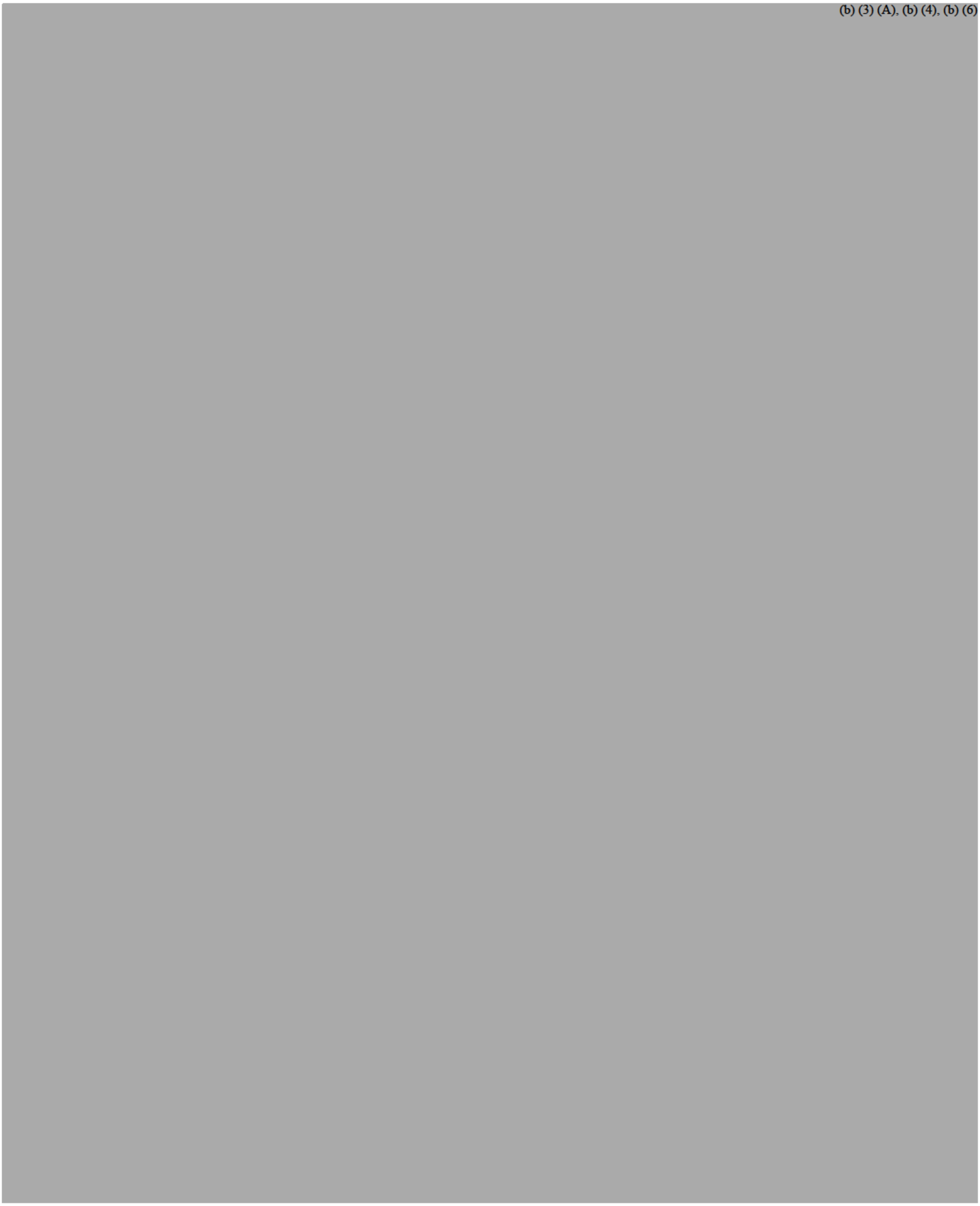
Animal:
All animals utilized in this proposal are housed in approved UTMB animal facilities that have been awarded accreditation by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC). Breeding and housing of AG129 and WT mice will be done in the MRB animal facility or in the GNL ABSL-2 laboratory. Detailed descriptions for housing, manipulations and handling of animals are described in the animal vertebrate section of this proposal.

Computer:
All lab personnel have access to personal computers, scanners and printers for data analysis, report preparation, and manuscript preparation. The University of Texas Medical Branch is fully networked locally and to the World Wide Web. For purposes of secure data storage, stand-alone, non-networked computers will be allocated as necessary. If necessary, those computers containing confidential information can be networked to one another, but in no case will these computers be accessible by anyone not directly involved in the project.

FACILITIES/RESOURCES AND MAJOR EQUIPMENT –

(b) (3) (A), (b) (4), (b) (6)

(b) (3) (A), (b) (4), (b) (6)


(b) (3) (A), (b) (4), (b) (6)


(b) (3) (A), (b) (4), (b) (6)

FACILITIES AND OTHER RESOURCES

(b) (3) (A), (b) (6)

Washington University School of Medicine

Laboratory:

(b) (3) (A) The laboratory contains an approved chemical fume hood and dedicated space for work with radioactivity, and provides bench space for at least 22 people. Additional BSL2 and BSL3 tissue culture rooms are across the hall and contain sterile hoods, incubators, microscopes, centrifuges, refrigerators, and freezers. BSL3 and A-BSL3 space has been certified by Washington University, the CDC, and the United States Department of Agriculture, specifically for work with different flaviviruses. The (b) (3) (A), laboratory also has approval from the Washington University Institutional Biosafety Committee for work with these viruses.

Animal:

Animals will be housed and cared for in a dedicated animal housing building. The facility includes work areas for animal manipulation procedures and containment facilities for ABSL3 level experiments. Full-time veterinarians monitor the health of the animals and, if necessary, provide appropriate treatment for illnesses and instructions in care.

Computer:

There are areas within the laboratory dedicated to computer-based work. The laboratory is equipped with a scanner, laser printers, fifteen Apple and PC computers linked by the Ethernet of Washington University, School of Medicine with direct access to GenBank, the World Wide Web and library facilities including subscriptions to electronic journals. Additional computers are dedicated to a real-time RT-PCR machine, BD-FACS Array flow cytometer, 96 well plate fluorimeter/spectrophotometer, and a T100 BIACORE instrument.

Other:

(b) (3) (A), (b) (6) has an office of approximately (b) (3) (A), (b) that is physically within the laboratory space. Additional desk space is provided in the laboratory for all members of the laboratory. Office capabilities include secretarial support, photocopying machines, a facsimile machine and personal computers. Subscriptions to pertinent journals are maintained in the office of the PI, and there is conference space dedicated for writing, meetings, and discussions. The meeting rooms have capacity for video- and computer conferencing.

Intellectual resources and collaborations:

In addition to the PI, the (b) (3) (A), laboratory consists of one Senior Research Associate Professor, two Instructors, eight postdoctoral investigators, and six graduate students. Laboratory members have diverse expertise including virology, immunology, cell biology, and structural biology. A collaborative research environment fosters multi-disciplinary studies along with other labs in the Departments of Microbiology and Pathology & Immunology at Washington University

Mentoring opportunities:

Interns, college, and medical students regularly perform research in the (b) (3) (A), laboratory, providing opportunities for more senior investigators to mentor and teach.

EQUIPMENT

(b) (3) (A), (b) (6)

Washington University School of Medicine

The (b) (3) (A), (b) (6) laboratory equipment includes heating and cooling water baths, shakers, balances, thermal cyclers, an ABI 7700 real-time PCR thermocycler, a TECAN 96-well plate ELISA reader and fluorimeter, an FPLC, two BD-FACS array flow cytometer with 96-well capacity, a T100 Biacore surface plasmon resonance instrument, microcentrifuges, table top centrifuges, ultracentrifuges, nucleic acid and protein electrophoresis apparatuses, high and low voltage power supplies, refrigerators and freezers, pH meters, inverted and fluorescence microscopes. The Departments of Pathology & Immunology and Molecular Microbiology and Division of Infectious Diseases have common facilities which include: constant temperature rooms at -20°, +4° and 37°C; photographic darkrooms, glassware washing and media preparation rooms; a fluorescence- activated cell sorter, an automatic X-ray film processor; confocal microscopes, electron microscopes, a BioRad phosphorimager, scintillation counters, and various microscopes and cameras. The Department of Pathology maintains hybridoma and histopathology core facilities as well as a LSR-II FACS sorter at BSL-3 capacity. There is a machine shop, an electronics shop, and automated DNA sequencing facilities. Finally, the Department of Medicine maintains protein chemistry, mass spectrometry, and biolayer interferometry facilities.

PRODUCT DEVELOPMENT STRATEGY

Milestones and Timeline

Introduction

As required by this RFA, outlined below are the key milestones and associated timelines for the Antiviral Drug Discovery and Development Center (AD3C) of which Dr. Richard Whitley is the Program Director. The theme is to *develop new small molecule therapeutics for emerging and re-emerging viral infections by inhibiting viral replication.* Activities will include the identification of novel chemical scaffolds and the application of basic virology research activities to identify and further probe the exact mechanism of action to identified lead molecules that inhibit viral replication. Medicinal chemistry and lead development will advance identified compounds through the drug discovery and development pathway, ultimately leading to preclinical evaluation of drug candidates to identify the most promising compounds for clinical evaluation. The AD3C has four interrelated research **Projects**, with a focus on coronaviruses, alphaviruses, flaviviruses and influenza. Each Project's efforts will be supported by three Cores: an Administrative Core (**Core A**), an Assay Core (**Core B**) and a Medicinal Chemistry and Lead Development Core (MCLDC; **Core C**). Organization and interaction between all Projects and Cores will be orchestrated by the Administrative Core, with day-to-day AD3C operations led by the Core's personnel. An Executive Committee (EC) consisting of all project and core leaders will regularly review the Projects' and Cores' activities and productivity. Finally, an external Scientific Advisory Committee (SAC) will be established to provide annually (with regular reports for '**Go-No go**' decisions) a high-level evaluation of AD3C's progress and successes, and aid in refining the Center's activities and direction. Unique to this proposal is that in addition to identifying lead compounds for particular viruses such as dengue or Zika virus, our approaches will identify 1) mechanisms and targets associated with viral replication; 2) assessment of resistance; 2) due to close interaction among Projects, compounds with activity against multiple viruses will be identified and could be pursued; and 3) analysis of the potential for combination therapies predicated on the use of nucleoside analog-based inhibition of viral polymerases paired with inhibitors of other steps in viral replication. These studies provide multiple opportunities to fulfill the goals outlined in the RFA.

Approach

The four projects working with the Assay Core, Medicinal Chemistry and Lead Development Core (MCLDC) and the Administrative Core will advance compounds through evaluation of compounds, medicinal chemistry, lead optimization and *in vivo* efficacy studies. Each Project will have the goal of identifying a lead series of compounds as well as a backup series to ensure success. The overarching goal is to find broad spectrum antiviral compounds for the treatment of emerging pathogens. The AD3C is uniquely qualified to do so, based upon the strategies outlined in the proposals, the team of scientists assembled, and the experience in collaborative drug discovery between Southern Research (SR), Gilead Sciences, the Emory Institute of Drug Discovery (EIDD), and respective scientists at the participating institutions. The basic science and mechanistic studies will be conducted by the four Projects, and the overall profile of what emerges will vary depending upon the results obtained. The three Cores will coordinate the evaluation, lead optimization, structure-activity relationship identification, pharmacokinetic evaluation (both *in vitro* and *in vivo*) and toxicology studies. The exact profile of the emerging lead compounds will vary and be driven by the scientific studies. However, to ensure that the AD3C can identify the compounds with the best overall profile and the greatest chance of succeeding, all of the data will be shared using the encrypted collaboration software, ShareFile.

Selection of an IND Candidate

The AD3C will utilize a pharmaceutical company model for discovery and development. In doing so we have established key attributes of a compound for advancing to an IND candidate, which we will use as a guide in our preclinical studies. These include:

Synthesis

- No structural alerts or reactive groups
- No issues in scale up for GLP toxicity and Phase I studies
- No impurity issues
- No significant stereochemistry issues

- No obvious cost of goods issues

Biopharmaceutics

- Crystalline, MP >125, non-hygro
- Bioavailability and oral PK completed with crystalline compound
- Solubility, PK and dose compatible with standard immediate release formulation
- Stability profile compatible with delivery options
- Required delivery options defined and realistic, according to disease target (i.e pneumonia versus encephalitis)

Absorption

- Conform with Lipinski's Rule of Five:
- Predicted human intestinal absorption will be adequate to achieve predicted efficacious human exposure and fraction absorbed ≥30%
- Exposure in dog or monkeys using single PO dose attaining exposure equivalent to predicted human clinical efficacious exposure

Clearance

- The predicted human PK for the compound will be assessed using in vitro parameters such as human liver microsome data and in vivo animal data
- Major CL mechanisms investigated in animals and predicted in humans including ID of likely CYPs and other metabolizing enzymes
- Variability in metabolic clearance assessed
- Projected human total CL < 10 ml/min/kg
- Projected oral bioavailability > 30%
- Metabolic Drug-Drug Interaction (DDI): Compound as cyp inhibitor assessed

PK/PD and Dose Prediction

- The *in vivo* relationship between drug concentration and pharmacodynamic response over time has been characterized and the human clinical pharmacology predictions relating to dose (frequency and amount) and onset, duration and magnitude of response fall within the criteria defined in the product profile

Distribution

- Plasma or serum protein binding compared in human, pharmacology, and tox species; blood to plasma ratio determined
- For CNS-targets only: not a substrate for P-gp.

Pharmacology

- Target Identified
- Defined Mechanism of Action
- Human target used for profiling
- The potential impact of human target polymorphisms has been considered.
- Potency differences between human target and species used in efficacy models is understood to guide human dose predictions

- Greater than 100x selectivity vs. relevant human targets

Safety

- Ames – negative *in vitro* test for bacterial mutagenicity
- Appropriate preclinical tox species selected
- Broad ligand profiling completed using standardized CEREP panel
- <50% inhibition of HERG, Nav1.5, LQT1, and L-type current at 100x the projected efficacious free-plasma levels in humans.

Intellectual Property

- Appropriate patent protection is in place for both the lead and back up series

These parameters will assist the AD3C to make decisions on an ongoing basis throughout the project. In addition, for this Project (flaviviruses), we have divided the drug discovery process up into six key measurable milestones and allocated the amount of time that will be needed to accomplish each goal (**Figure 1**). These timelines were established based upon past experience and industry standards and are set to identify an IND candidate(s). However, a large portion of the funds required for the late stage preclinical and IND enabling GLP studies are not available as through this RFA, and additional funds will be sought.

Key Milestones

1. Hit and Lead identification (11 months)
2. Primary lead compounds identified (11 months)
3. *In vivo* efficacy with proof of concept compound (7 months)
4. Preclinical Candidate (14 months)
5. IND Enabling Studies – Candidate Selection (12 months)
6. IND Preparation (3 months)

Each of these milestones has a number of associated studies and decision points that are listed below and outlined in Figure 1.

Details

1) Hit Identification (11 months)
    a. HTS of nucleoside sub-libraries (EIDD, (b) (3) (A), validated and completed
    b. Selectivity in secondary assays
        i. Compounds with potency (EC_{50}<1μM) and no cytotoxicity (>10 fold EC_{50})
    c. Chemistry triage identifies multiple chemical series with potency and selectivity
    d. Preliminary structure-activity demonstrated
2) Primary lead compounds identified. Previously identified non-nucleoside inhibitors enter at this point in development (11 months)
    a. Potency of < 0.5 μM against viral replication
    b. Tractable synthesis
    c. Oral bioavailability of >30% in mice
    d. No activity at 10 μM in a general receptor profiling panel
    e. Synthetic feasibility determined (availability of starting materials, number of steps, timelines to produce 25 -50 grams) and freedom to operate analysis completed
3) *In vivo* efficacy with proof of concept compound (7 months)
    a. Lead series selected with cell-based activity
    b. Back-up series identified
    c. Robust structure activity demonstrated, mechanism understood, potency

d. No hERG activity
e. Solubility (≥50 μM), microsomal stability (>60% remaining at 30 minutes), measureable plasma free fractions (>1%) and an assessment of permeability (CaCo2)
f. Bioavailability such that plasma concentration is 5-10 fold above EC_{50}
g. Oral bioavailability of >30% in rodents preferred

4) Preclinical Candidate (14 months)
   a. *In vitro* antiviral activity and selectivity. Ranges for antiviral potency would be typically EC_{50} < 0.1 μM and CC_{50} > 20 μM in cell culture.
   b. Structure-activity relationships versus a primary endpoint indicating antiviral activity or mechanism
   c. Testing in a broad receptor profile including a set of key cardiac and CNS ion channels and receptors (no activity at 10 μM)
   d. Compound stability as a dry powder and in solution at a range of pH's
   e. No inhibition of cytochrome P450's at 10 μM (3A4, 2C9,2C19)
   f. Expanded PK studies including a second species (preferably dog). A plasma half-life of >2 h (mouse or rat) and >30% bioavailability. Metabolite identification in vivo.
   g. Oral activity in a relevant mouse model of infection. No observable *in vivo* side-effects. CNS exposure if relevant (ED_{50} of <100 mg/Kg)
   h. Understanding the metabolic pathways involved using rodent, dog and human microsomes and hepatocytes as well as *in vivo* in rodents and dogs prior to toxicology studies
   i. Chemistry feasibility and cost of goods analysis, which will be key as larger quantities of compound (100-200 grams) would be needed for some of the preliminary studies
   j. A 7-day non-GLP toxicity study in rodents (Determination of No Effect Level (NOEL) and/or No Adverse Effect Level (NOAEL))
   k. Predicted human dose of ≤500 mg per day.

5) Candidate Selection (12 months) IND Enabling Studies
   a. GMP Manufacture of 1Kg of drug substance
      i. Stability studies to support toxicity
   b. Formulation development
   c. 14-day GLP toxicity study in rodents and dogs
   d. Cardiovascular assessment

6) IND Preparation (3 months) will be done at UAB, as past experience has demonstrated

These timelines are estimates and can vary depending upon compounds availability, resources and capacity at the GMP manufacturing facilities. The majority of these studies will be conducted with Southern Research's GLP facilities.

ID	Task Name	Start	Finish
1	**Flavivirus**	**Fri 3/1/19**	**Thu 2/29/24**
2	**Aim 1. Develop Preclinical DAAs Targeting Flavivirus Capsid and NS4B Protein**	**Fri 3/1/19**	**Thu 6/1/23**
3	1.1 Establish SAR for DENV C inhibitor	Fri 3/1/19	Tue 8/31/21
4	1.2 Determine co-crystal structures of C and inhibitor	Sat 6/1/19	Mon 2/28/22
5	1.3 Determine EC50 and CC50 of C and NS4B inhibitors	Sun 9/1/19	Mon 2/28/22
6	1.4 Analysis of additional novel inhibitors	Fri 3/1/19	Mon 2/28/22
7	1.5 Resistance analysis and antiviral spectrum	Sun 9/1/19	Tue 5/31/22
8	1.6 Analyze PK/PD of C and NS4B inhibitors	Sun 9/1/19	Thu 6/1/23
9	1.7 Determine mouse efficacy	Sun 3/1/20	Thu 6/1/23
10	**Aim 2. Identify Novel Nuc DAAs with Pan-Favivirus Activity**	**Fri 3/1/19**	**Thu 8/31/23**
11	2.1 Perform HTS of Nuc libraries	Fri 3/1/19	Sat 2/29/20
12	2.2 MedChem prodrug	Mon 3/2/20	Tue 2/28/23
13	2.3 Determine EC50, CC50, and toxicity	Mon 3/2/20	Tue 2/28/23
14	2.4 Resistance analysis and antiviral spectrum	Tue 9/1/20	Tue 2/28/23
15	2.5 Determine mouse efficacy	Tue 9/1/20	Thu 8/31/23
16	**Aim 3. Evaluate combination therapy directed against flavivirus infection**	**Mon 3/1/21**	**Thu 2/29/24**
17	3.1 Perform combination efficacy in cells	Mon 3/1/21	Tue 2/28/23
18	3.2 Perform resistance analysis	Mon 3/1/21	Tue 2/28/23
19	3.3 Determine EC50 and CC50	Tue 6/1/21	Tue 2/28/23
20	3.4 Determine mouse efficacy	Tue 6/1/21	Thu 8/31/23
21	3.5 Determine NHP efficacy	Wed 3/1/23	Thu 2/29/24

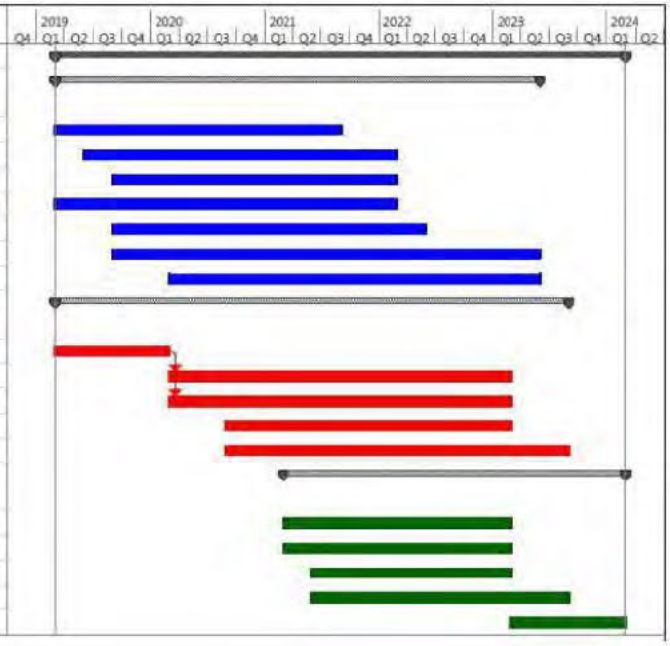


Figure 1 Timeline Project 3

Product Development Plan

Introduction

The goal of this proposal is to develop direct antiviral agents (DAAs) for flavivirus therapy. We will focus initially on compounds that inhibit dengue and Zika viruses with emphasis on compounds that inhibit replication of both viruses, other members of the flavivirus family, and potentially members of other virus families as well. To do so, a series of **Go/No-Go** decisions have been established with the goal of obtaining (an) orally active drug(s). Described below are the criteria required to identify lead compounds suitable for animal model testing, the selection of a preclinical compound with the efficacy and safety profile to advance into non-GLP studies, additional metabolic profiling and pharmacokinetic studies. Synthetic feasibility will also be considered. These studies will enable the team to select a compound for IND enabling GLP studies leading to a phase I clinical candidate(s).

1. *Intended use/indication of the proposed product and the biodefense/public health gap the product is intended to fill*

This project will focus on the development of drugs to treat dengue virus and Zika virus infection. These viruses are both mosquito-borne members of the flavivirus family, and both represent emerging public health threats in the U.S. and worldwide. The dengue viruses are classified as NIAID category A, whereas ZIKV was added to the NIAID priority pathogen list in 2016 and classified as an "additional emerging pathogen." Currently, no antiviral therapies are available for the treatment of either virus – let alone West Nile virus. DENV comprises four related but antigenically distinct serotypes (each of which has multiple virus genotypes) that are associated with dengue fever as well as more severe disease, including dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). As described in the Significance of Project 3, Dengue is endemic in much of the tropical and subtropical regions of the globe. Worldwide, the annual number of dengue infections is estimated to be 390 million, with 500,000 cases of DHF/DSS. Dengue virus infection typically presents as an acute febrile illness characterized by headache, acute joint and muscle pain, and leukopenia. Severe forms of the disease vascular leakage and hypovolemic shock. Epidemic outbreaks of DENV have been reported in many countries throughout Latin America, South-East Asia, and the Western Pacific Regions. DENV also are prevalent in Puerto Rico and periodically cause autochthonous outbreaks in the continental US, including a recent large outbreak in Key West Florida (>5.4% incidence) in 2009-2010. Currently there is no specific antiviral therapy to treat DENV infection. A vaccine licensed in several counties, Dengvaxia, has proven only 30-60% efficacious and has been associated with adverse outcomes in DENV naïve individuals, suggesting that the need for antiviral drugs may not be diminished by vaccination in the near future.

ZIKV has emerged explosively as a major public health threat. Like DENV, ZIKV is primarily transmitted by *Aedes* species mosquitoes. In addition, ZIKV can infect humans through a sexual route, blood transfusion, organ transplantation, and maternal-fetal transmission. ZIKV initially was isolated from a sentinel rhesus macaque in 1947 in the Zika Forest of Uganda. Until 2007, ZIKV had circulated silently in many parts of Africa and Asia without causing severe disease or large outbreaks, with fewer than 20 documented human infections. During the recent epidemics, ZIKV infection has caused devastatingly severe diseases, including congenital malformations in the fetus of infected pregnant women (microcephaly and fetal demise) and Guillain-Barré syndrome in adults. Phylogenetic analysis revealed that ZIKV evolved into African and Asian lineages. Strains from the Asian lineage are responsible for the recent large-scale epidemics on the Yap Island in 2007, in the French Polynesia and South Pacific in 2013, and in the Americas in 2015 and 2016. In the past three years, ZIKV vaccine development has progressed rapidly, including inactivated, subunit, and live-attenuated vaccines. Several ZIKV vaccine candidates have entered or completed phase I clinical trials. Compared with vaccine development, progress on ZIKV therapeutics has lagged. Although a number of laboratories have identified small molecular inhibitors (primarily through screening FDA-approved drugs) and therapeutic antibodies, none has entered clinical trials[39]

We intend to use the existing platforms that have been developed at Southern Research (SR), UTMB (Pei-Yong Shi), Washington University (b) (3) (A), (b) (6) and OHSU (b) (3) (A), (b) (6)) to focus on

drugs that directly target viral proteins. This include nucleoside analog inhibitors of the viral RNA-dependent RNA polymerase (RdRp), as well non-nucleoside inhibitors of DENV capsid, ZIKV NS4B, and others that have been identified in by the earlier work of this consortium. Our intent is to discover molecules that are active at different viral proteins and, therefore, offer the opportunity for combination.

2. *Statement of the value of the project, including lay description of key technology objectives, innovation and advantages compared to competing products, technologies or services*

The value of this project is the contribution of drugs to treat DENV and ZIKV infection, and potentially other flaviviruses. In essence, the value of the project resides in the multidisciplinary approach of an experienced team of scientists with expertise in molecular biology, drug discovery, evaluation of antiviral activity, medicinal chemistry, animal models of viral pathogenesis, preclinical compound evaluation and, ultimately, the translation of our molecule discovery program into clinical development if the opportunity presents. The key technological objectives are the application of a seamless approach to rapid drug development. Lead molecules for the inhibition of DENV and ZIKV replication have been identified that provide a technological starting point for product development. While other groups may pursue the discovery of lead molecules, such as those noted above, we have a distinct advantage of the availability of world class experts in viral biology and medicinal chemistry being an integral component of our application.

3. *Description of the goal(s) of the project, including one (or more) intermediate products, final product(s) or stage(s) of product development to be completed during the award period. (no specific product profile by licensing indication is needed)*

As noted above, the primary goal of the project is to develop drugs to treat flavivirus infection. We already have established several of the needed assays and have lead molecules that can be used to initiate mechanism of action studies with the opportunity for performing proof of principle early in this grant. In addition, we have obtained well-characterized capsid and NS4B inhibitors that are undergoing lead optimization. Our goal is to identify additional hits and leads within a limited collection of nucleoside analogs and improve the spectrum of activity and efficacy of these lead products. Furthermore, mechanistic studies will be performed. A detailed research plan has been established with clear criteria for compound advancement. Using a **Go/No-Go** decision tree plan (see below under point 5) we attempt to finalize two to three molecules that will provide a choice for a preclinical development pathway leading to the selection of one or more clinical candidates, including the potential for combination.

4. *A detailed description of the qualitative and quantitative criteria and data elements to be used to assess the scientific merit of feasibility of proceeding to the next stage of development*

As a key aspect of their responsibilities our external Scientific Advisory Committee and the Executive Committee will work in a coordinated fashion to guarantee both prioritization and productivity of each project. As was learned in the current CETR, ongoing decisions regarding continuation or discontinuation will be required.

On a more granular level, the research efforts and approaches leading to the selection of lead compounds are described in Project 3 as well as in the Assay and Medicinal Chemistry and Lead Development Core descriptions. The goal is to identify lead compounds and eventually clinical candidates for the treatment of flavivirus infection. In summary, the lead compounds identified will have demonstrated *in vivo* efficacy in two or more infectious animal models when administered orally. In addition the compound's pharmacokinetic profile (half-life and bioavailability, distribution) would need to indicate that human doses of < 500 mg once or twice a day would be sufficient. Any compound progressing to this point would have had preliminary pharmacokinetic studies completed and have oral bioavailability in mice or rats of ≥30% and a minimum half-life of approximately 2 h. Several additional studies would then be done to advance the compound further. CNS exposure will be determined, and distribution studies will be required. For treatment of WNV or other neurotropic flaviviruses, entry of the drug into the CNS is, a desirable characteristic. Once we have established oral bioavailability, we will move to animal models to test efficacy (mouse, non-human primate). In addition, if the *in vitro* data warrants, these compounds would be tested across a panel of different viruses.

In parallel, we will perform:

- Testing in a broad receptor profile including a set of key cardiac and CNS ion channels and receptors
- Compound stability as a dry powder and in solution at a range of pH's
- Examining the compound's effect on cytochrome P450's will be required as some of these compounds will be used in combination with other drugs
- Expanded PK studies including a second species (preferably dog, for which SR has the capability)
- Metabolite identification *in vivo*
- Chemistry feasibility and cost of goods analysis, which will be key as larger quantities of compound (100-200 grams) would be needed for some of the preliminary studies
- A 7-day non-GLP toxicity study to determine a maximum tolerated dose, identify any toxicities and establish doses for the GLP toxicity study (Determination of No Effect Level (NOEL) and/or No Adverse Effect Level (NOAEL))

Most lead optimization programs produce multiple lead compounds, typically two to four, and require an additional series of pre-GLP studies to select the compound with the best profile. The decision tree to select the drug candidate will be based upon the collection of properties of the compounds including efficacy, safety profile, physical properties (stability, solubility, crystal forms, etc.), pharmacokinetics, ease and cost of synthesis, dosage and formulation.

Monthly conference calls of the Executive Committee will review ongoing data. At least annually, the external Scientific Advisory Committee will meet to review the drug development plan. The NIH project officer for this grant will be invited to attend the yearly meetings to guide us regarding NIH partnerships that could facilitate product development. As we approach the identification of a clinical candidate, the external Scientific Advisory Committee will be able to assist the decision-making process.

From these targeted meetings, molecules will be selected for further development. Once a preclinical candidate is identified, it will be tested in a fairly standard GLP program to collect the appropriate data for an IND filing. Initially, chemistry would involve synthetic route optimization to provide sufficient GMP API to support the GLP preclinical studies. In parallel to this, further optimization would be performed to provide sufficient API for phase I/II clinical studies. Depending on the route of administration (oral delivery is preferred), formulation and stability studies would be conducted to optimize these routes (oral or IV, other options). Ideally, the compound will have the appropriate bioavailability and half-life for oral administration. Therefore, the Phase I studies will be conducted with a powder in a bottle formulation. The powder-in-bottle (PIB) approach will be used to accelerate development and introduction into Phase I clinical trials. SR has the appropriate expertise to facilitate this PIB strategy. This approach consists of compounding the active pharmaceutical ingredient (API) into a solution or a suspension in the clinic prior to oral administration. The development will consist of physico-chemical assessment, constitution fluid selection, weighing and dosing validation, and stability evaluation of API, before and after constitution with the fluid. This approach will provide a high degree of dosing flexibility during the initial phase of clinical trials. Additionally, the PIB approach will reduce the time and API required for clinical development and supplies to < 2 months and < 100 gm, respectively.

A general GLP toxicity package would be developed. Full Ames and Mouse Lymphoma genotoxicity studies would be conducted. This would include acute oral and intravenous studies in rat and/or mouse and another species, typically dog for 28 days. The choice of species will depend in part upon the efficacy animal models. The length of the studies will be determined based upon the phase I/II clinical plan. A GLP safety pharmacology package would be generated that could include rat, dog (or primates depending on species selections above), Erwin studies and cardiovascular safety studies in 2 species and Perkinje Fiber electrophysiology. After IND filing, long term genotoxicity studies as well as longer term animal safety studies would be initiated. Additional funding for these studies would be required.

5. *A description of the distinct stages/activities that are gates for Go/No Go decisions for advancement*

From the set of experiments and capabilities outlined in the project proposal as well as in the Assay and Medicinal Chemistry and Lead Development Cores, compounds will be identified with the following criteria:

1) In vitro antiviral activity and selectivity versus normal cells. Ranges for antiviral potency would be typically EC_{50} < 0.1 μM and CC_{50} > 20 μM in cell culture.
2) Structure-activity relationships versus a primary endpoint indicating antiviral activity or mechanism
3) Solubility (≥50 μM), microsomal stability (>60% remaining at 30 minutes), measureable plasma free fractions (>1%) and an assessment of permeability (CaCo2).
4) No inhibition of cytochrome 3A4 or 2C9 at 10 μM.
5) A plasma half-life of >2 h (mouse or rat) and >30% bioavailability.
6) Oral activity in a relevant mouse model of infection. No observable *in vivo* side-effects. CNS exposure if relevant (*e.g.*, WNV).
7) Understanding the metabolic pathways involved using both rodent, dog and human microsomes as well as *in vivo* in rodents and dogs prior to toxicology studies.
8) Tissue distribution
9) Synthetic feasibility determined (availability of starting materials, number of steps, timelines to produce 25 -50 grams) and freedom to operate analysis completed

In summary, a preclinical compound would be orally active in a mouse model with dosing of ≤10 mg/kg and based upon the in vitro activity and pharmacokinetic profile the projected human dose would be <500 mg/day. A compound meeting these criteria from any of the projects would be designated a preclinical candidate and thus advance to a series of FDA regulated GLP studies required for IND submission.

The key milestones for the GLP studies and IND submission include:

1) GMP manufacturing of 500 -1000 grams of API to be used for GLP toxicity studies and formulation development. On average this can take approximately 6 months.
2) Identification of a second species for GLP toxicity studies. Typically dogs, but non-human primates may offer some advantages for certain indications. Once initiated the GLP toxicity studies will take about 6-9 months to complete the in vivo studies and generate the reports.
3) Preparation of the IND

6. *The performance specs/features the product should have in order to provide therapeutic benefit*

- Small molecule (MW <500), orally bioavailable
- No more than twice a day administration
- No more than 5-14 day treatment course
- Absence of bone marrow suppression or hepatic toxicity.
- Absence of development limiting adverse events (seizures in an animal model)
- Tissue distribution to target organs

7. *A description and developmental status of the assays for the product release and characterization, including activity and efficacy (this may not be needed per se; depends on the stage of drug development)*

All of the assays for the performance of activity and efficacy determinations as well as initial characterization of the compounds are within the capability of this application at either the participating institutions or in the Screening and Medicinal Chemistry and Lead Development Cores. These include *in vitro* screens of activity, mechanisms to develop resistant virus, monoclonal antibodies to assess drug distribution, and well characterized animal models. Furthermore, we have the resources of Southern Research to perform GLP clinical pharmacology and all preclinical toxicology required for an Investigational New Drug application.

The certification, characterization, stability and release parameters of the GMP drug substance (API) will be conducted by an established GMP manufacturing company. The drug product development, stability and release will be contracted to an established Drug Product manufacturer. (b) (3) (A), (b) (6) has extensive experience with this

process. He was responsible for the Drug Substance and Drug Product for two IND's; (b) (3) (A), (b) (6), as well as a Phase 3 compound, (b) (3) (A), (b) (6). For example, with (b) (3) (A), (b) (6), he was responsible for all of the IND CMC documentation for drug product and drug substance. Kilogram quantities of GMP API were prepared using a Contract Research Organization. The material was then sent to another facility where the initial powder-in-a-bottle formulation was developed and used in the Phase I clinical trials, followed by a tablet formulation used in the subsequent phase 2 trials.

8. *Discussion with the FDA, if any, relevant to the development activities of the candidate product.*

We envision involving the FDA on an informal basis (if allowed) in order to expedite drug development. This has worked most successfully for the PI (Richard Whitley) in studies performed by the NIAID Collaborative Antiviral Study Group. To be specific, the NIAID CASG has had joint discussions with the Antiviral Division of the FDA involving unmet medical need and the proper clinical pathways to meet these needs. Toward this end, the Antiviral Branch of the FDA may have specific issues that we will need to address in this application.

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: Dr. First Name*: PEI-YONG Middle Name Last Name*: SHI Suffix: Ph.D

Position/Title*: I.H. Kempner Professor of Human Genetics
Organization Name*: The University of Texas Medical Branch at Galveston
Department: BMB
Division:
Street1*: 5.136 Medical Research Building (b) (3) (A),
Street2: University of Texas Medical Branch
City*: Galveston
County:
State*: TX: Texas
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 775551055

Phone Number*: 409-772-6370 Fax Number:

E-Mail*: peshi@utmb.edu

Credential, e.g., agency login: (b) (6)

Project Role*: Other (Specify) Other Project Role Category: Project Lead

Degree Type: PHD Degree Year: 1995

Attach Biographical Sketch*: File Name: Shi_NIHBio_Feb_28_2018.pdf

Attach Current & Pending Support: File Name:

Tracking Number: GRANT12598083 Funding Opportunity Number: RFA-AI-17-042 . Received Date: 2018-03-29T16:30:43.000-04:00

PROFILE - Senior/Key Person

Prefix: First Name*: (b) (3) (A), (b) (6) Middle Name: (b) (3) (A), Last Name*: (b) (3) (A), (b) (6) Suffix: Ph.D

Position/Title*: Professor and Director
Organization Name*: Oregon Health & Science University
Department: Vaccine/Gene Therapy Institute
Division:
Street1*: 505 NW 185th Ave, Mail Code VGTI
Street2:
City*: Beaverton
County: Washington
State*: OR: Oregon
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 97006-3448

Phone Number*: (b) (3) (A), (b) (6) Fax Number: (b) (3) (A), (b) (6)

E-Mail*: (b) (3) (A), (b) (6)

Credential, e.g., agency login: (b) (6)

Project Role*: Co-Investigator Other Project Role Category:

Degree Type: PhD Degree Year: (b) (3) (A), (b)

Attach Biographical Sketch*: File Name: Biosketch_(b) (3) (A), (b) (6).pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: First Name*: (b) (3) (A), (b) (6) Middle Name: (b) (3) (A), (b) (6) Last Name*: (b) (3) (A), (b) (6) Suffix: Ph.D

Position/Title*: Assistant Professor
Organization Name*: Oregon Health & Science Unversity
Department: Vaccine/Gene Therapy Institute
Division:
Street1*: 505 NW 185th Ave, Mail Code VGTI
Street2:
City*: Beaverton
County: Washington
State*: OR: Oregon
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 97006-3448

Phone Number*: (b) (3) (A), (b) (6) Fax Number: (b) (3) (A), (b) (6)

E-Mail*: (b) (3) (A), (b) (6)

Credential, e.g., agency login: (b) (6)

Project Role*: Co-Investigator Other Project Role Category:

Degree Type: PhD Degree Year: (b) (3) (A), (b)

Attach Biographical Sketch*: File Name: Biosketch_(b) (3) (A), (b) (6).pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix:	
First Name*:	(b) (3) (A) (b)
Middle Name	(b
Last Name*:	(b) (3) (A),
Suffix:	
Position/Title*:	Professor
Organization Name*:	Washington University
Department:	Infectious Diseases
Division:	School of Medicine
Street1*:	WASHINGTON UNIVERSITY
Street2:	660 S. Euclid Ave
City*:	SAINT LOUIS
County:	
State*:	MO: Missouri
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	631100000
Phone Number*:	(b) (3) (A), (b) (6)
Fax Number:	(b) (3) (A), (b) (6)
E-Mail*:	(b) (3) (A), (b) (6)
Credential, e.g., agency login:	(b) (6)
Project Role*:	Co-Investigator
Other Project Role Category:	
Degree Type:	MD, PhD
Degree Year:	(b) (3) (A), (b)
Attach Biographical Sketch*:	File Name: Biosketch (b) (3) (A), .pdf
Attach Current & Pending Support:	File Name:

Tracking Number: GRANT12598083 Funding Opportunity Number: RFA-AI-17-042 . Received Date: 2018-03-29T16:30:43.000-04:00

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 0636907050000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2019 **End Date*:** 02-29-2020 **Budget Period:** 1

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Pei-Yong		Shi		PD/PI	(b) (6), (b) (4)	(b) (6)			0.00	0.00	0.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **0.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**0.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-17-042. Received Date: 2018-03-29T16:30:43.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 0636907050000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2019 **End Date*:** 02-29-2020 **Budget Period: 1**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 0636907050000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2019 **End Date*:** 02-29-2020 **Budget Period: 1**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	1,035,067.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**1,035,067.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**1,035,067.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. Modified Total Direct on 1st 25,000 of 2 subawards	48.5	75,000.00	36,375.00
		Total Indirect Costs	**36,375.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number) DHHS Shon Turner 214 767-3261

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**1,071,442.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	1,071,442.00

L. Budget Justification* File Name: Budget_Justification_Flavi_Overall.pdf

(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Tracking Number: GRANT12598083 Funding Opportunity Number: RFA-AI-17-042. Received Date: 2018-03-29T16:30:43.000-04:00

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 0636907050000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2019 **End Date*:** 02-28-2021 **Budget Period: 2**

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Pei-Yong		Shi		PD/PI		(b) (6)			0.00	0.00	0.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **0.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**0.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-17-042. Received Date: 2018-03-29T16:30:43.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 0636907050000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2019 **End Date*:** 02-28-2021 **Budget Period: 2**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 0636907050000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2019 **End Date*:** 02-28-2021 **Budget Period: 2**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	1,040,903.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**1,040,903.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**1,040,903.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
		Total Indirect Costs	

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**1,040,903.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	1,040,903.00

L. Budget Justification* File Name: Budget_Justification_Flavi_Overall.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Tracking Number: GRANT12598083 Funding Opportunity Number: RFA-AI-17-042. Received Date: 2018-03-29T16:30:43.000-04:00

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 0636907050000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2019 **End Date*:** 02-28-2022 **Budget Period: 3**

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Pei-Yong		Shi		PD/PI		(b) (6)			0.00	0.00	0.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **0.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**0.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-17-042. Received Date: 2018-03-29T16:30:43.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 0636907050000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2019 **End Date*:** 02-28-2022 **Budget Period: 3**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 0636907050000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2019 **End Date*:** 02-28-2022 **Budget Period: 3**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	1,046,664.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**1,046,664.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**1,046,664.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
		Total Indirect Costs	

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**1,046,664.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	1,046,664.00

L. Budget Justification* File Name: Budget_Justification_Flavi_Overall.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Tracking Number: GRANT12598083 Funding Opportunity Number: RFA-AI-17-042. Received Date: 2018-03-29T16:30:43.000-04:00

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 0636907050000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2019 **End Date*:** 02-28-2023 **Budget Period: 4**

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Pei-Yong		Shi		PD/PI		(b) (6)			0.00	0.00	0.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **0.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**0.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 0636907050000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2019 **End Date*:** 02-28-2023 **Budget Period: 4**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 0636907050000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2019 **End Date*:** 02-28-2023 **Budget Period: 4**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	1,051,820.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**1,051,820.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**1,051,820.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
		Total Indirect Costs	

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**1,051,820.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	1,051,820.00

L. Budget Justification* File Name: Budget_Justification_Flavi_Overall.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Tracking Number: GRANT12598083 Funding Opportunity Number: RFA-AI-17-042. Received Date: 2018-03-29T16:30:43.000-04:00

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 0636907050000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2019 **End Date*:** 02-29-2024 **Budget Period: 5**

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Pei-Yong		Shi		PD/PI		(b) (6)			0.00	0.00	0.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **0.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**0.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 0636907050000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2019 **End Date*:** 02-29-2024 **Budget Period: 5**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 0636907050000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2019 **End Date*:** 02-29-2024 **Budget Period: 5**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	1,141,185.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**1,141,185.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**1,141,185.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
		Total Indirect Costs	

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**1,141,185.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	1,141,185.00

L. Budget Justification*	File Name: Budget_Justification_Flavi_Overall.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Tracking Number: GRANT12598083 Funding Opportunity Number: RFA-AI-17-042. Received Date: 2018-03-29T16:30:43.000-04:00

BUDGET JUSTIFICATION PROJECT 3 FLAVIVIRUS

UAB has the prime CETR award; the Administrative Core will administer the subawards to the three project sites involved with Project 3. Please find the budget justification for those sites under their respective subaward budgets.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		0.00
Section B, Other Personnel		0.00
Total Number Other Personnel	0	
Total Salary, Wages and Fringe Benefits (A+B)		0.00
Section C, Equipment		0.00
Section D, Travel		0.00
1. Domestic	0.00	
2. Foreign	0.00	
Section E, Participant/Trainee Support Costs		0.00
1. Tuition/Fees/Health Insurance	0.00	
2. Stipends	0.00	
3. Travel	0.00	
4. Subsistence	0.00	
5. Other	0.00	
6. Number of Participants/Trainees	0	
Section F, Other Direct Costs		5,315,639.00
1. Materials and Supplies	0.00	
2. Publication Costs	0.00	
3. Consultant Services	0.00	
4. ADP/Computer Services	0.00	
5. Subawards/Consortium/Contractual Costs	5,315,639.00	
6. Equipment or Facility Rental/User Fees	0.00	
7. Alterations and Renovations	0.00	
8. Other 1	0.00	
9. Other 2	0.00	
10. Other 3	0.00	
Section G, Direct Costs (A thru F)		5,315,639.00
Section H, Indirect Costs		36,375.00
Section I, Total Direct and Indirect Costs (G + H)		5,352,014.00
Section J, Fee		0.00
Section K, Total Direct and Fee (I + J)		5,352,014.00

Tracking Number: GRANT12598083 Funding Opportunity Number: RFA-AI-17-042. Received Date: 2018-03-29T16:30:43.000-04:00

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 8007711490000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: The University of Texas Medical Branch at Galveston

Start Date*: 03-01-2019 **End Date*:** 02-29-2020 **Budget Period:** 1

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		Pei-Yong		Shi		Project Co-Leader	(b) (6), (b) (4)	(b) (6)			28,050.00	4,034.00	32,084.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **32,084.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
2	Post Doctoral Associates	(b) (6)			95,442.00	33,854.00	129,296.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
2	**Total Number Other Personnel**					**Total Other Personnel**	**129,296.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**161,380.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 8007711490000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: The University of Texas Medical Branch at Galveston

Start Date*: 03-01-2019 **End Date*:** 02-29-2020 **Budget Period: 1**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs		Funds Requested ($)*
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 8007711490000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: The University of Texas Medical Branch at Galveston

Start Date*: 03-01-2019 **End Date*:** 02-29-2020 **Budget Period: 1**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	38,500.00
2. Publication Costs	3,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**41,500.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**205,880.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. UTMB F&A Base	58.0	205,880.00	119,410.00
		Total Indirect Costs	**119,410.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number)	DHHS, Division of Cost Allocation Arif Karim, Director Phone: 214-767-9861

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**325,290.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	325,290.00

L. Budget Justification*	File Name: Budget_Justification_Mar_2_2018_Shi.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Tracking Number: GRANT12598083 Funding Opportunity Number: RFA-AI-17-042. Received Date: 2018-03-29T16:30:43.000-04:00

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 8007711490000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: The University of Texas Medical Branch at Galveston

Start Date*: 03-01-2020 **End Date*:** 02-28-2021 **Budget Period: 2**

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Pei-Yong		Shi		Project Co-Leader	(b) (6), (b) (4)	(b) (6)			28,050.00	4,034.00	32,084.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **32,084.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
2	Post Doctoral Associates	(b) (6)			95,442.00	33,854.00	129,296.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
2	**Total Number Other Personnel**					**Total Other Personnel**	**129,296.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**161,380.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 8007711490000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: The University of Texas Medical Branch at Galveston

Start Date*: 03-01-2020 **End Date*:** 02-28-2021 **Budget Period: 2**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 8007711490000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: The University of Texas Medical Branch at Galveston

Start Date*: 03-01-2020 **End Date*:** 02-28-2021 **Budget Period: 2**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	38,500.00
2. Publication Costs	3,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**41,500.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**205,880.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. UTMB F&A Base	58.0	205,880.00	119,410.00
		Total Indirect Costs	**119,410.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number)	DHHS, Division of Cost Allocation Arif Karim, Director Phone: 214-767-9861

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**325,290.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	325,290.00

L. Budget Justification*	File Name: Budget_Justification_Mar_2_2018_Shi.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Tracking Number: GRANT12598083 Funding Opportunity Number: RFA-AI-17-042. Received Date: 2018-03-29T16:30:43.000-04:00

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 8007711490000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: The University of Texas Medical Branch at Galveston

Start Date*: 03-01-2021 **End Date*:** 02-28-2022 **Budget Period: 3**

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Pei-Yong		Shi		Project Co-Leader	(b) (6), (b) (4)	(b) (6)			28,050.00	4,034.00	32,084.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **32,084.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
2	Post Doctoral Associates	(b) (6)			95,442.00	33,854.00	129,296.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
2	**Total Number Other Personnel**					**Total Other Personnel**	**129,296.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**161,380.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 8007711490000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: The University of Texas Medical Branch at Galveston

Start Date*: 03-01-2021 **End Date*:** 02-28-2022 **Budget Period: 3**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs		Funds Requested ($)*
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 8007711490000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: The University of Texas Medical Branch at Galveston

Start Date*: 03-01-2021 **End Date*:** 02-28-2022 **Budget Period: 3**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	38,500.00
2. Publication Costs	3,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**41,500.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**205,880.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. UTMB F&A Base	58.0	205,880.00	119,410.00
		Total Indirect Costs	**119,410.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number)	DHHS, Division of Cost Allocation Arif Karim, Director Phone: 214-767-9861

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**325,290.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	325,290.00

L. Budget Justification*	File Name: Budget_Justification_Mar_2_2018_Shi.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Tracking Number: GRANT12598083 Funding Opportunity Number: RFA-AI-17-042. Received Date: 2018-03-29T16:30:43.000-04:00

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 8007711490000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: The University of Texas Medical Branch at Galveston

Start Date*: 03-01-2022 **End Date*:** 02-28-2023 **Budget Period: 4**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		Pei-Yong		Shi		Project Co-Leader	(b) (6), (b) (4)	(b) (6)			28,050.00	4,034.00	32,084.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **32,084.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
2	Post Doctoral Associates	(b) (6)			95,442.00	33,854.00	129,296.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
2	**Total Number Other Personnel**					**Total Other Personnel**	**129,296.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**161,380.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-17-042. Received Date: 2018-03-29T16:30:43.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 8007711490000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: The University of Texas Medical Branch at Galveston

Start Date*: 03-01-2022 **End Date*:** 02-28-2023 **Budget Period: 4**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs		Funds Requested ($)*
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 8007711490000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: The University of Texas Medical Branch at Galveston

Start Date*: 03-01-2022 **End Date*:** 02-28-2023 **Budget Period: 4**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	38,500.00
2. Publication Costs	3,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**41,500.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**205,880.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. UTMB F&A Base	58.0	205,880.00	119,410.00
		Total Indirect Costs	**119,410.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number)	DHHS, Division of Cost Allocation Arif Karim, Director Phone: 214-767-9861

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**325,290.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	325,290.00

L. Budget Justification*	File Name: Budget_Justification_Mar_2_2018_Shi.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Tracking Number: GRANT12598083 Funding Opportunity Number: RFA-AI-17-042. Received Date: 2018-03-29T16:30:43.000-04:00

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 8007711490000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: The University of Texas Medical Branch at Galveston

Start Date*: 03-01-2023 **End Date*:** 02-29-2024 **Budget Period: 5**

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Pei-Yong		Shi		Project Co-Leader	(b) (6), (b) (4)	(b) (6)			18,700.00	2,689.00	21,389.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **21,389.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
2	Post Doctoral Associates	(b) (6)			47,722.00	16,926.00	64,648.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
2	**Total Number Other Personnel**					**Total Other Personnel**	**64,648.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**86,037.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 8007711490000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: The University of Texas Medical Branch at Galveston

Start Date*: 03-01-2023 **End Date*:** 02-29-2024 **Budget Period: 5**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs		Funds Requested ($)*
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 8007711490000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: The University of Texas Medical Branch at Galveston

Start Date*: 03-01-2023 **End Date*:** 02-29-2024 **Budget Period: 5**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	18,750.00
2. Publication Costs	3,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**21,750.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**110,787.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. UTMB F&A Base	58.0	110,787.00	64,256.00
		Total Indirect Costs	**64,256.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number)	DHHS, Division of Cost Allocation Arif Karim, Director Phone: 214-767-9861

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**175,043.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	175,043.00

L. Budget Justification*	File Name: Budget_Justification_Mar_2_2018_Shi.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Tracking Number: GRANT12598083 Funding Opportunity Number: RFA-AI-17-042. Received Date: 2018-03-29T16:30:43.000-04:00

Budget justifications

Pei-Yong Shi, PhD, PI, will devote (b) (6) calendar months and receive (b) (3) (A), calendar months salary support. Dr. Shi is a recognized leader in the field of flavivirus drug discovery and flavivirus biology. He used to serve as Executive Director at Novartis Institute for Tropical Diseases to set up anti-infective strategy and execute drug discovery and development. He will oversee the flavivirus project and work closely with (b) (3) (A), (b) (6), and other team members to deliver the proposed milestones. His team at UTMB will perform DENV capsid crystal structure in complex with inhibitor, ZIKV NS4B inhibitor analysis, and Nuc characterization. In addition, together with other key members of the project, he will generate progress reports and manuscripts.

(b) (3) (A), (b) (6) Ph.D. Post-doctoral fellow. Will devote (b) (3) calendar months and receive (b) (3) calendar months salary support. (b) (3) (A), (b) (6) has a strong track record in flavivirus NS4B biology and drug discovery. (b) (3) identified dengue viral NS4B dimerization, NS3/NS4B interaction, and NS4A/NS4B interaction. (b) (3) (A), will perform all experiments related to NS4B inhibitors as well as nucleoside inhibitors. (b) (3) (A), has expertise in biochemistry, virology, and animal model.

(b) (3) (A), (b) (6), Ph.D. Post-doctoral fellow. Will devote (b) (3) calendar months and receive (b) (3) calendar months salary support. (b) (3) (A), (b) will be responsible for all experiments related to DENV capsid inhibitors. He has solved the co-crystal structure of DENV-2 capsid protein in complex with ST-148 inhibitors. (b) (3) (A), (b) has expertise in both biochemistry and molecular virology. He will test compound efficacy, cytotoxicity, and resistance analysis.

Reagents: $23,500 per year
PCR reagents, PCR purification kits, plasmid purification kits, cell culture medium, cell culture flasks, electroporation reagents, antibodies, restriction enzymes for DNA cloning, DNA sequencing

Mouse: $15,000 per year
AG129 mice, cage charges, and other animal related costs.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		149,725.00
Section B, Other Personnel		581,832.00
Total Number Other Personnel	10	
Total Salary, Wages and Fringe Benefits (A+B)		731,557.00
Section C, Equipment		0.00
Section D, Travel		15,000.00
1. Domestic	15,000.00	
2. Foreign	0.00	
Section E, Participant/Trainee Support Costs		0.00
1. Tuition/Fees/Health Insurance	0.00	
2. Stipends	0.00	
3. Travel	0.00	
4. Subsistence	0.00	
5. Other	0.00	
6. Number of Participants/Trainees	0	
Section F, Other Direct Costs		187,750.00
1. Materials and Supplies	172,750.00	
2. Publication Costs	15,000.00	
3. Consultant Services	0.00	
4. ADP/Computer Services	0.00	
5. Subawards/Consortium/Contractual Costs	0.00	
6. Equipment or Facility Rental/User Fees	0.00	
7. Alterations and Renovations	0.00	
8. Other 1	0.00	
9. Other 2	0.00	
10. Other 3	0.00	
Section G, Direct Costs (A thru F)		934,307.00
Section H, Indirect Costs		541,896.00
Section I, Total Direct and Indirect Costs (G + H)		1,476,203.00
Section J, Fee		0.00
Section K, Total Direct and Fee (I + J)		1,476,203.00

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 0969975150000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Oregon Health and Science University

Start Date*: 03-01-2019 **End Date*:** 02-29-2020 **Budget Period:** 1

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		(b) (3) (A), (b) (6)			Ph.D	Project Co-Leader	(b) (6), (b) (4)	(b) (6)			18,700.00	4,675.00	23,375.00
2.					Ph.D	Project Co-Investigator					34,036.00	8,509.00	42,545.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **65,920.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Resrch Asst Professor	(b) (6)			23,897.00	10,037.00	33,934.00
1	Research Associate				17,945.00	5,383.00	23,328.00
1	Research Assistant 2				23,465.00	5,866.00	29,331.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**86,593.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**152,513.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 0969975150000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Oregon Health and Science University

Start Date*: 03-01-2019 **End Date*:** 02-29-2020 **Budget Period: 1**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 0969975150000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Oregon Health and Science University

Start Date*: 03-01-2019 **End Date*:** 02-29-2020 **Budget Period: 1**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	31,500.00
2. Publication Costs	1,500.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**33,000.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**188,513.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. MTDC	54.0	188,513.00	101,797.00
		Total Indirect Costs	**101,797.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Arif M. Karim, 214-767-3600

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**290,310.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	290,310.00

L. Budget Justification* File Name: (b) (3) (A), (b) (6) Project_3_UAB_budget_justif_FINAL-2.pdf

(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Tracking Number: GRANT12598083 Funding Opportunity Number: RFA-AI-17-042. Received Date: 2018-03-29T16:30:43.000-04:00

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 0969975150000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Oregon Health and Science University

Start Date*: 03-01-2020 **End Date*:** 02-28-2021 **Budget Period: 2**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.				(b) (3) (A), (b) (6)	Ph.D	Project Co-Leader	(b) (6), (b) (4)	(b) (6)			18,700.00	4,675.00	23,375.00
2.					Ph.D	Project Co-Investigator					35,057.00	8,764.00	43,321.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **67,196.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Resrch Asst Professor	(b) (6)			24,614.00	10,338.00	34,952.00
1	Research Associate				18,483.00	5,545.00	24,028.00
1	Research Assistant 2				24,169.00	6,042.00	30,211.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**89,191.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**156,387.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 0969975150000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Oregon Health and Science University

Start Date*: 03-01-2020 **End Date*:** 02-28-2021 **Budget Period: 2**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Tracking Number: GRANT12598083 Funding Opportunity Number: RFA-AI-17-042. Received Date: 2018-03-29T16:30:43.000-04:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 0969975150000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Oregon Health and Science University

Start Date*: 03-01-2020 **End Date*:** 02-28-2021 **Budget Period: 2**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	31,500.00
2. Publication Costs	1,500.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**33,000.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**192,387.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. MTDC	54.0	192,387.00	103,889.00
		Total Indirect Costs	**103,889.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Arif M. Karim, 214-767-3600

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**296,276.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	296,276.00

L. Budget Justification* File Name: (b) (3) (A), (b) (6) Project_3_UAB_budget_justif_FINAL-2.pdf

(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Tracking Number: GRANT12598083 Funding Opportunity Number: RFA-AI-17-042. Received Date: 2018-03-29T16:30:43.000-04:00

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 0969975150000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Oregon Health and Science University

Start Date*: 03-01-2021 **End Date*:** 02-28-2022 **Budget Period: 3**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		(b) (3) (A), (b) (6)			Ph.D	Project Co-Leader	(b) (6), (b) (4)	(b) (6)			18,700.00	4,675.00	23,375.00
2.					Ph.D	Project Co-Investigator					36,108.00	9,027.00	45,135.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **68,510.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Resrch Asst Professor	(b) (6)			25,353.00	10,648.00	36,001.00
1	Research Associate				19,038.00	5,711.00	24,749.00
1	Research Assistant 2				24,894.00	6,223.00	31,117.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**91,867.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**160,377.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 0969975150000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Oregon Health and Science University

Start Date*: 03-01-2021 **End Date*:** 02-28-2022 **Budget Period: 3**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 0969975150000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Oregon Health and Science University

Start Date*: 03-01-2021 **End Date*:** 02-28-2022 **Budget Period: 3**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	31,500.00
2. Publication Costs	1,500.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**33,000.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**196,377.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. MTDC	54.0	196,377.00	106,044.00
		Total Indirect Costs	**106,044.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Arif M. Karim, 214-767-3600

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**302,421.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	302,421.00

L. Budget Justification* File Name: (b) (3) (A), (b) (6)-Project_3_UAB_budget_justif_FINAL-2.pdf

(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Tracking Number: GRANT12598083 Funding Opportunity Number: RFA-AI-17-042. Received Date: 2018-03-29T16:30:43.000-04:00

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 0969975150000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Oregon Health and Science University

Start Date*: 03-01-2022 **End Date*:** 02-28-2023 **Budget Period: 4**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		(b) (3) (A), (b) (6)			Ph.D	Project Co-Leader	(b) (6), (b) (4)	(b) (6)			18,700.00	4,675.00	23,375.00
2.					Ph.D	Project Co-Investigator					37,192.00	9,298.00	46,490.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **69,865.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Resrch Asst Professor	(b) (6)			26,113.00	10,966.00	37,079.00
1	Research Associate				19,609.00	5,883.00	25,492.00
1	Research Assistant 2				25,641.00	6,410.00	32,051.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**94,622.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**164,487.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 0969975150000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Oregon Health and Science University

Start Date*: 03-01-2022 **End Date*:** 02-28-2023 **Budget Period: 4**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 0969975150000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Oregon Health and Science University

Start Date*: 03-01-2022 **End Date*:** 02-28-2023 **Budget Period: 4**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	30,500.00
2. Publication Costs	1,500.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**32,000.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**199,487.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. MTDC	54.0	199,487.00	107,723.00
		Total Indirect Costs	**107,723.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Arif M. Karim, 214-767-3600

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**307,210.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	307,210.00

L. Budget Justification* File Name: (b) (3) (A), (b) (6)Project_3_UAB_budget_justif_FINAL-2.pdf

(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Tracking Number: GRANT12598083 Funding Opportunity Number: RFA-AI-17-042. Received Date: 2018-03-29T16:30:43.000-04:00

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 0969975150000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Oregon Health and Science University

Start Date*: 03-01-2023 **End Date*:** 02-29-2024 **Budget Period: 5**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		(b) (3) (A), (b) (6)			Ph.D	Project Co-Leader	(b) (6), (b) (4)	(b) (6)			18,700.00	4,675.00	23,375.00
2.					Ph.D	Project Co-Investigator					38,307.00	9,577.00	47,384.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **71,259.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Resrch Asst Professor	(b) (6)			26,896.00	11,297.00	38,193.00
1	Research Associate				20,197.00	6,059.00	26,256.00
1	Research Assistant 2				26,410.00	6,602.00	33,012.00
1	Veterinary Pathologist				9,350.00	2,338.00	11,688.00
2	Senior Research Asst				11,088.00	4,255.00	15,343.00
6	**Total Number Other Personnel**					**Total Other Personnel**	**124,492.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**195,751.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 0969975150000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Oregon Health and Science University

Start Date*: 03-01-2023 **End Date*:** 02-29-2024 **Budget Period: 5**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 0969975150000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: Oregon Health and Science University

Start Date*: 03-01-2023 **End Date*:** 02-29-2024 **Budget Period: 5**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	42,500.00
2. Publication Costs	1,500.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. NHP Lease Fees and Per Diems	170,738.00
9. NHP Necropsy, Procedures	56,003.00
Total Other Direct Costs	**270,741.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**469,492.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. MTDC	54.0	215,721.00	116,489.00
2. MTDC	75.0	253,772.00	190,329.00
		Total Indirect Costs	**306,818.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number) DHHS, Arif M. Karim, 214-767-3600

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**776,310.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	776,310.00

L. Budget Justification* File Name: (b) (3) (A), (b) (6) Project_3_UAB_budget_justif_FINAL-2.pdf

(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

BUDGET JUSTIFICATION – OREGON HEALTH & SCIENCE UNIVERSITY

PERSONNEL ($829,515)

(b) (3) (A), (b) (6), **PhD, Subcontract Principal Investigator** (b) (6) effort, (b) (6) cal. months). (b) (3) (A), (b) (6) will provide the oversight and organizational structure to ensure clear lines of authority and execution of research activities, including those of all key personnel and technical staff. (b) (3) (A), (b) (6) will have the responsibility for planning and maintaining communication between the subcontractor team and contractor as well as management of the subcontract's resources. He will also have the primary role in promoting scientific interactions, research progress, and facilitating communications between key personnel and the NIH. The PI will be responsible for ensuring compliance with all institutional, federal, and NIH regulations as well as the safety and security of data, materials, facilities and resources of the program.

(b) (3) (A), (b) (6), **PhD, Co-Investigator** (b) (6) effort, (b) (6) cal. months). (b) (3) (A), (b) (6) is an Assistant Professor at the Vaccine and Gene Therapy Institute (VGTI) and will serve as Co-Investigator of the OHSU sub-contract. (b) (3) (A) possesses extensive experience with *in vitro* and *in vivo* models of flavivirus infection including nonhuman primates. Additionally, he has helped drive the early development of several of the anti-viral compounds described in this study. (b) (3) (A), (b) (6) will focus on further development of flavivirus inhibitors previously identified at Southern Research. During SAR-based optimization studies, he will coordinate efforts with the medicinal chemistry core to improve compound efficacy and pharmacological properties. In the final year of the proposal, (b) (3) (A), (b) (6) will supervise nonhuman primate studies on the most promising drugs or drug combinations.

(b) (3) (A), (b) (6), **PhD, Research Assistant Professor** (b) (6) effort, (b) (6) cal. months). (b) (3) (A), (b) (6) is an experienced researcher who has studied multiple aspects of flavivirus-host interactions. (b) (3) has worked with multiple anti-flavivirus compounds and has studied multiple aspects of flavivirus-host cell interactions. (b) (3) will be responsible for conducting secondary and tertiary screens in conjunction with (b) (3) (A), (b) (6) as well as conducting follow-up studies characterizing mechanisms of compound action.

(b) (3) (A), (b) (6), **MS, Research Associate** (b) (6) effort, (b) (6) cal. months). (b) (3) (A), (b) (6) is a technician in the (b) (3) (A), (b) (6) laboratory. He will be responsible for producing titered stocks of viruses for infection studies and assisting as needed with antiviral assays. He will also be responsible for processing of macaque samples and virus quantification.

(b) (3) (A), (b) (6), **MS, Research Assistant 2** (b) (6) effort, (b) (6) cal. months). (b) (3) (A), (b) (6) is a technician in the (b) (3) (A), (b) (6) laboratory. He will be responsible for producing titered stocks of viruses for infection studies and assisting as needed with antiviral assays. He will also be responsible for processing of macaque samples and virus quantification.

(b) (3) (A), (b) (6), **D.V.M., Ph.D., Veterinary Pathologist** (Year 5: (b) (6) effort, (b) (6) calendar months). (b) (3) (A) is a veterinary pathologist with a specialty in infectious disease with over (b) (3) years of experience investigating mechanisms of viral pathogenesis in nonhuman primate (NHP) models. He will supervise the Animal Research Unit to provide support for all NHP studies proposed in this application. (b) (3) (A), (b) (6) has extensive experience conducting NHP studies at the ABSL-2/3 and -3 levels.

(b) (3) (A), (b) (6), **Senior Research Assistant** (Year 5: (b) (6) effort, (b) (6) calendar months). (b) (3) (A), (b) (6) is an experienced member of the Infectious Disease Resource team who will perform NHP inoculations, obtain blood samples, and make daily health observations.

(b) (3) (A), (b) (6), **Senior Research Assistant** (Year 5: (b) (6) effort, (b) (6) calendar months). (b) (3) (A), (b) (6) is an experienced member of the Infectious Disease Resource team who will perform NHP inoculations, obtain blood samples, and make daily health observations

ANIMAL COSTS ($170,738)

The Division of Comparative Medicine (DCM) is responsible for providing optimum standards of care for all animals used in research programs. Animal care includes identification, housing and feeding of incoming animals, proper and regular sanitation of cages, and adequate veterinary care including control and diagnosis of diseases, etc. Expenses incurred for utilizing NHP from the ONPRC include:

Rhesus macaque lease fees: In year 5, lease fees for 18 adult rhesus macaques will cost $156,353 ($8,686.27 each).

NHP Per Diems: 18 animals will spend up to 44 days in Tier 2 indoor housing ($12.67/day) during PK studies with compounds and 14 days in Tier 2 containment ($17.26/day). Time in tier 1 allows for multiple PK studies if additional dose regimens need to be assessed. Also allows for training of animals to use blood collection tower, abrogating need for sedation during blood draw. Total requested for all animals: $14,385

OTHER EXPENSES (Animal procedures: $56,003)
Fees for procedures and surgical services are provided by the ONPRC Division of Comparative Medicine staff.

NHP Necropsies: We are requesting Grade 3 complex necropsies with histology and up to 60 slides to fully examine the distribution of virus and immune/inflammatory cells in the infected NHP. All animals will go to necropsy following ZIKV challenge in Year 5. Total: $48,758 for all animals ($2,708.75/animal x 18 animals).

NHP Blood Sample Collection: In Year 5, we are requesting a total of $2,967 ($11.77/blood draw) for a total of 252 blood draws for 18 animals.

NHP Urine Sample Collection: In Year 5, we are requesting a total of $1,907 ($11.77/urine sample collection) for a total of 162 urine sample collections for 18 animals.

Clinical Test Chem Panel: In Year 5, we are requesting a total of $506 for 18 Clinical Test Chem Panels ($28.12/test) for each animal.

Clinical Test CBC: In Year 5, we are requesting a total of $263 for 18 Clinical Test CBCs ($14.60/test).

Clinical Test: DIFF: Year 5, $432. Differential blood count to examine relative white blood cell count following drug administration for 18 Clinical Test DIFFs ($24/test).

Drug Administration: Year 5, $1,172. Drug administration and viral challenge injections; Includes drug administration during PK studies. Total = 156 injections ($7.51/administration).

OTHER COSTS

PUBLICATIONS ($7,500)
Results will be published in peer-reviewed journals as they are satisfactorily completed. ($1,500/yr Years 1-5)

MATERIALS AND SUPPLIES ($167,500)
Antibodies: Virus specific antibodies for detection of detection of flavivirus replication in focus forming assays, as well as detection of viral proteins by immunofluorescence and western blotting. Additionally, includes secondary antibodies for focus assays, westerns, and IF assays. ($5,000/yr Years 1-5)

Molecular biology supplies: These will be required for all molecular biological and biochemical experimentation, including qPCR (for viral quantification), immunoblotting (to monitor antibody specificity), immunofluorescence, and viral replication monitoring/evaluation. This includes enzymes (e.g., Taq polymerase) and chemicals (e.g., SDS, TRIS base, agarose, TEMED, Tween, BSA, formalin, ethanol, DMSO), agar, and LB broth. ($8,000/yr Years 1-4, $20,000 Year 5, due to extensive RNA isolation and qRT-PCR for viral quantification)

Tissue culture supplies: These will be required for all cell growth and maintenance as well as virus growth and titration and isolation from tissues. This includes cell culture growth media, animal serum, PBS, trypsin, sucrose, sorbitol, disposable sterilizing filters, antibiotics, and syringes. ($8,000/yr Years 1-5)

Plasticware/ glassware: Disposable plasticware will be required for cell and virus culture, virus titration and virus isolation, and molecular biological work. This includes tissue culture dishes of diverse sizes and layouts, flasks, serological pipettes, disposable pipette tips, microfuge and centrifuge tubes, and disposable screw cap tubes of various sizes for sample storage. ($8,000/yr Years 1-3, $7,000/yr Years 4-5)

LN2 and CO2: will be required for all tissue culture. Liquid nitrogen will be required for cryopreservation of all primary cells and cell lines. ($500/yr Years 1-5)

Personal protective equipment (PPE): gloves, gowns, masks, protective eyeware for tissue culture and virus work. ($1,000/yr Years 1-5)

Cell viability assays: Cell titer-glo or equivalent assays for determination of *in vitro* cellular toxicity of experimental antiviral compounds. ($1,000/yr Years 1-5)

TRAVEL ($15,000)

Travel costs have been allocated for one scientific meeting per year for (b) (3) (A), (b) (6). This will be necessary to communicate results obtained from the proposed studies and to stay current on related outside research activities and protocols. Funds in the amount of $3,000 annually for Years 1-5 are requested for round-trip airfare, per diems (meals and lodging), meeting registration and miscellaneous travel expenses (parking, ground transportation) based on up to four nights of lodging and five days of per diem. The daily maximum amount for meals and lodging may vary, based on location in high-cost areas.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		342,750.00
Section B, Other Personnel		486,765.00
Total Number Other Personnel	18	
Total Salary, Wages and Fringe Benefits (A+B)		829,515.00
Section C, Equipment		0.00
Section D, Travel		15,000.00
1. Domestic	15,000.00	
2. Foreign	0.00	
Section E, Participant/Trainee Support Costs		0.00
1. Tuition/Fees/Health Insurance	0.00	
2. Stipends	0.00	
3. Travel	0.00	
4. Subsistence	0.00	
5. Other	0.00	
6. Number of Participants/Trainees	0	
Section F, Other Direct Costs		401,741.00
1. Materials and Supplies	167,500.00	
2. Publication Costs	7,500.00	
3. Consultant Services	0.00	
4. ADP/Computer Services	0.00	
5. Subawards/Consortium/Contractual Costs	0.00	
6. Equipment or Facility Rental/User Fees	0.00	
7. Alterations and Renovations	0.00	
8. Other 1	170,738.00	
9. Other 2	56,003.00	
10. Other 3	0.00	
Section G, Direct Costs (A thru F)		1,246,256.00
Section H, Indirect Costs		726,271.00
Section I, Total Direct and Indirect Costs (G + H)		1,972,527.00
Section J, Fee		0.00
Section K, Total Direct and Fee (I + J)		1,972,527.00

Tracking Number: GRANT12598083 Funding Opportunity Number: RFA-AI-17-042. Received Date: 2018-03-29T16:30:43.000-04:00

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 0685522070000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Washington University

Start Date*: 03-01-2019 **End Date*:** 02-29-2020 **Budget Period:** 1

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		(b) (3) (A), (b) (6)				Project Co-Leader	(b) (6), (b) (4)	(b) (6)			13,090.00	2,828.00	15,918.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **15,918.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	(b) (6)			49,887.00	15,212.00	65,099.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Sr. Staff Scientist				17,699.00	5,535.00	23,234.00
1	Technician				8,439.00	3,170.00	11,609.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**99,942.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**115,860.00**

RESEARCH & RELATED Budget (A-B) (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 0685522070000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Washington University

Start Date*: 03-01-2019 **End Date*:** 02-29-2020 **Budget Period: 1**

C. Equipment Description		
List items and dollar amount for each item exceeding $5,000		
Equipment Item		**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file		
	Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 0685522070000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Washington University

Start Date*: 03-01-2019 **End Date*:** 02-29-2020 **Budget Period: 1**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	106,500.00
2. Publication Costs	2,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Mouse Per Diem, Mouse Breeders, Pharmacy and Dissection, BSL costs, equipment service cost	47,700.00
Total Other Direct Costs	**156,200.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**275,060.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. MTDC	52.5	275,060.00	144,407.00
		Total Indirect Costs	**144,407.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number)	DHHS, Division of Cost Allocation, 1301 Young Street, Dallas, TX 75202, 214-767-3261

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**419,467.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	419,467.00

L. Budget Justification*	File Name: (b) (3) (A), (b) (6)_CETR_Whitley_Budget_Justifucatiion_copy.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Tracking Number: GRANT12598083 Funding Opportunity Number: RFA-AI-17-042. Received Date: 2018-03-29T16:30:43.000-04:00

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 0685522070000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Washington University

Start Date*: 03-01-2020 **End Date*:** 02-28-2021 **Budget Period: 2**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.				(b) (3) (A), (b) (6)		Project Co-Leader	(b) (6), (b) (4)	(b) (6)			13,090.00	2,884.00	15,974.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **15,974.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
		(b) (6)					
1	Post Doctoral Associates				51,383.00	16,599.00	67,982.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Sr. Staff Scientist				18,229.00	5,959.00	24,188.00
1	Technician				8,693.00	3,638.00	12,331.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**104,501.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**120,475.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-17-042. Received Date: 2018-03-29T16:30:43.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 0685522070000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Washington University

Start Date*: 03-01-2020 **End Date*:** 02-28-2021 **Budget Period: 2**

C. Equipment Description		
List items and dollar amount for each item exceeding $5,000		
Equipment Item		**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file		
	Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 0685522070000
Budget Type*: ○ Project ● Subaward/Consortium
Enter name of Organization: Washington University

Start Date*: 03-01-2020 **End Date*:** 02-28-2021 **Budget Period: 2**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	102,500.00
2. Publication Costs	2,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Mouse Per Diem, Mouse Breeders, Pharmacy and Dissection, BSL costs, equipment service cost	47,000.00
Total Other Direct Costs	**151,500.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**274,975.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. MTDC	52.5	274,975.00	144,362.00
		Total Indirect Costs	**144,362.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number)	DHHS, Division of Cost Allocation, 1301 Young Street, Dallas, TX, 75202, 214-767-3261.

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**419,337.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	419,337.00

L. Budget Justification* File Name: (b) (3) (A), (b) (6)CETR_Whitley_Budget_Justifucatiion_copy.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Tracking Number: GRANT12598083 Funding Opportunity Number: RFA-AI-17-042. Received Date: 2018-03-29T16:30:43.000-04:00

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 0685522070000

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: Washington University

Start Date*: 03-01-2021 **End Date*:** 02-28-2022 **Budget Period: 3**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.				(b) (3) (A), (b) (6)		Project Co-Leader	(b) (6), (b) (4)	(b) (6)			13,090.00	2,927.00	16,017.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **16,017.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	(b) (6)			52,925.00	17,845.00	70,770.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Sr. Staff Scientist				18,776.00	6,340.00	25,116.00
1	Technician				8,953.00	3,867.00	12,820.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**108,706.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**124,723.00**

RESEARCH & RELATED Budget (A-B) (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 0685522070000
Budget Type*: ❍ Project ● Subaward/Consortium
Enter name of Organization: Washington University

Start Date*: 03-01-2021 **End Date*:** 02-28-2022 **Budget Period: 3**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs		Funds Requested ($)*
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 0685522070000
Budget Type*: ❍ Project ● Subaward/Consortium
Enter name of Organization: Washington University

Start Date*: 03-01-2021 **End Date*:** 02-28-2022 **Budget Period: 3**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	102,500.00
2. Publication Costs	2,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Mouse Per Diem, Mouse Breeders, Pharmacy and Dissection, BSL costs, equipment service cost	42,500.00
Total Other Direct Costs	**147,000.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**274,723.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. MTDC	52.5	274,723.00	144,230.00
		Total Indirect Costs	**144,230.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number)	DHHS, Division of Cost Allocation, 1301 Young Street, Dallas, TX, 75202, 214-767-3261.

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**418,953.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	418,953.00

L. Budget Justification* File Name: (b) (3) (A), (b) (6)CETR_Whitley_Budget_Justifucatiion_copy.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Tracking Number: GRANT12598083 Funding Opportunity Number: RFA-AI-17-042. Received Date: 2018-03-29T16:30:43.000-04:00

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 0685522070000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Washington University

Start Date*: 03-01-2022 **End Date*:** 02-28-2023 **Budget Period: 4**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		(b) (3) (A), (b) (6)				Project Co-Leader	(b) (6), (b) (4)	(b) (6)			13,090.00	2,959.00	16,049.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **16,049.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	(b) (6)			54,513.00	19,022.00	73,535.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Sr. Staff Scientist				19,340.00	6,738.00	26,078.00
1	Technician				9,222.00	4,080.00	13,302.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**112,915.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**128,964.00**

RESEARCH & RELATED Budget (A-B) (Funds Requested)

Funding Opportunity Number: RFA-AI-17-042. Received Date: 2018-03-29T16:30:43.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 0685522070000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Washington University

Start Date*: 03-01-2022 **End Date*:** 02-28-2023 **Budget Period: 4**

C. Equipment Description		
List items and dollar amount for each item exceeding $5,000		
Equipment Item		**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file		
	Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 0685522070000
Budget Type*: ❍ Project ● Subaward/Consortium
Enter name of Organization: Washington University

Start Date*: 03-01-2022 **End Date*:** 02-28-2023 **Budget Period: 4**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	101,000.00
2. Publication Costs	2,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Mouse Per Diem, Mouse Breeders, Pharmacy and Dissection, BSL costs, equipment service cost	40,000.00
Total Other Direct Costs	**143,000.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**274,964.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. MTDC	52.5	274,964.00	144,356.00
		Total Indirect Costs	**144,356.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number)	DHHS, Division of Cost Allocation, 1301 Young Street, Dallas, TX, 75202, 214-767-3261.

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**419,320.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	419,320.00

L. Budget Justification* File Name: (b) (3) (A), (b) (6)CETR_Whitley_Budget_Justifucatiion_copy.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Tracking Number: GRANT12598083 Funding Opportunity Number: RFA-AI-17-042. Received Date: 2018-03-29T16:30:43.000-04:00

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 0685522070000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Washington University

Start Date*: 03-01-2023 **End Date*:** 02-29-2024 **Budget Period: 5**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.			(b) (3) (A), (b) (6)			Project Co-Leader	(b) (6), (b) (4)	(b) (6)			13,090.00	2,985.00	16,075.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **16,075.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
		(b) (6)					
1	Post Doctoral Associates				28,075.00	10,086.00	38,161.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Technician				3,166.00	1,428.00	4,594.00
2	**Total Number Other Personnel**					**Total Other Personnel**	**42,755.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**58,830.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-17-042. Received Date: 2018-03-29T16:30:43.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 0685522070000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Washington University

Start Date*: 03-01-2023 **End Date*:** 02-29-2024 **Budget Period: 5**

C. Equipment Description		
List items and dollar amount for each item exceeding $5,000		
Equipment Item		**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file		
	Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Tracking Number: GRANT12598083 Funding Opportunity Number: RFA-AI-17-042. Received Date: 2018-03-29T16:30:43.000-04:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 0685522070000
Budget Type*: ❍ Project ● Subaward/Consortium
Enter name of Organization: Washington University

Start Date*: 03-01-2023 **End Date*:** 02-29-2024 **Budget Period: 5**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	46,000.00
2. Publication Costs	2,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Mouse Per Diem, Mouse Breeders, Pharmacy and Dissection, BSL costs, equipment service cost	14,650.00
Total Other Direct Costs	**62,650.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**124,480.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. MTDC	52.5	124,480.00	65,352.00
		Total Indirect Costs	**65,352.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number)	DHHS, Division of Cost Allocation, 1301 Young Street, Dallas, TX, 75202, 214-767-3261.

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**189,832.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	189,832.00

L. Budget Justification* File Name: (b) (3) (A), (b) (6)CETR_Whitley_Budget_Justifucatiion_copy.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Tracking Number: GRANT12598083 Funding Opportunity Number: RFA-AI-17-042. Received Date: 2018-03-29T16:30:43.000-04:00

BUDGET JUSTIFICATION

(b) (3) (A), (b) (6)

Washington University School of Medicine

PERSONNEL:

(b) (3) (A), (b) (6), **M.D., Ph.D. Principal Investigator.** Professor of Medicine, Molecular Microbiology, Pathology and Immunology at Washington University School of Medicine. (b) (3) (A), (b) (6) will devote (b) (6) calendar months to this project and receive (b) (6) calendar months of salary support. He will be responsible for the design and oversight of all investigations occurring in (b) (3) laboratory. He will actively participate in the planning and execution of experiments, as well as in the generation of progress reports and manuscripts.

(b) (3) (A), (b) (6), **Ph.D. Research Associate.** (b) (3) (A), is a post-doctoral associate who is working full-time on Zika virus therapeutic project. He has significant experience in evaluating flavivirus pathogenesis and analyzing correlates of protection in vivo. He will devote (b) calendar months (b) (6) effort) of time to this project in FY1-4 and 6 months FY5 and derive commensurate salary from it. He will be responsible for performing many of the *in vivo* experiments with the lead compounds and analyzing virological and pathological endpoints. He will also help interpret data and troubleshoot technical problems in consultation with the principal investigator.

(b) (3) (A), (b) (6), **Ph.D. Staff Scientist.** (b) (3) (A), (b) is a staff scientist in the laboratory with more than (b) decades of experience in animal models of disease. (b) will devote (b) calendar months (b) (6) effort) of time to this project and derive (b) calendar months of salary from it in FY1-4. (b) will help (b) (3) (A), with many of the *in vivo* studies given the scale of the project. (b) also will help interpret data and troubleshoot technical problems in consultation with the principal investigator.

(b) (3) (A), (b) (6), **Animal Technician.** This project requires a significant amount of animal work associated with breeding and conducting animal experiments. (b) (3) (A), our experienced animal technician, will be responsible for animal husbandry, and also perform some infection experiments, under the oversights of (b) (3) (A), (b) (6) (b) will devote (b) calendar months to the project and respectively receive salary support in FY1-4, with a smaller effort in FY5.

SUPPLIES:

Tissue culture ($20,000). With this project, there will be a considerable amount of tissue culture. The funds requested will be used for media preparation, serum, antibiotics, plasticware (disposable pipets, pipetman tips, flasks, tubes, cryogenic vials, filtration flasks, sterile bottles), cytokine supplements, and hybridoma and antibody growth. In particular, defined serum ($300/bottle), growth factor supplements, primary cell cultures, and are expensive to propagate. In addition, we will need to grow large stocks of viruses and titrate tissue homogenates for viral yield.

Molecular Biology Reagents ($12,500). This amount has been budgeted for reagents for molecular cloning (restriction enzymes), proteases, transfection (liposomes, electroporation cuvettes), plasmid DNA and RNA purification kits, mutagenesis, vectors, bacterial culture supplies, and DNA/RNA/protein electrophoresis. This will be especially important for many of the mechanism of action studies with antibodies.In addition, some of these funds will be used for a sensitive and reproducible quantitative real-time RT-PCR for assessing alphavirus replication using an ABI 7700 Sequence Detection instrument. This assay has become our standard for RNA quantitation and will be used to measure viral RNA levels in serum and in tissues. RT-PCR reagents, primers, TaqMan probes cost approximately $1 per well. In addition, for most samples, a ribosomal or actin RNA control is run for normalization purposes.

General ($15,000). This amount has been budgeted for general chemical supplies including buffers, salts, organic solvents, acids, bases, and detergents, as well as radiochemicals and disposal, when needed. We also will purchase oligonucleotides that will be used for cloning and sequencing. Alos, these funds will be used for purchasing small equipment (pipetteman (multi- single- serological), vortexes, waterbaths, microcentrifuges) and disposable tubes and tips. .Finally, this amount includes funds for liquid N2/CO2 bottled gas and related tank rental fees, and glasswashing,

MAR1-5A3 anti-Ifnar1 antibodies ($7,000). To generate lethal challenge models of ZIKV mice are administered a 2 mg dose of MAR1-5A3. This will be used in many of our protection models. MAR1-5A3 will be purchased

commercially. We expect to used ~1 g of mAb per year for the challenge studies, which can be purchased from Leinco Technologies.

Immunochemical Reagents ($17,000). This has been budgeted for the direct labeling of multiple antibodies with different fluorophores, for secondary reagents for ELISA, and for intracellular immunofluorescence studies. We also will use part of these funds for analysis of purified MAbs for *in vivo* studies. Finally, this amount is for reagents associated with running of the flow cytometer including calibration, buffers, controls, and software licenses. Some of our high-throughput analyis will utilize flow-cytometry based readouts. In addition, we will use this equipment for analyzing viral infection in different tissues.

Animal purchase or cost ($35,000). This cost reflects the purchase of WT C57BL/6 mice or the cost of producing human STAT2-KI mice from our own breeding colony. The latter is more expensive given their smaller litter sizes. In addition, some of the studies will be performed in pregnant animals, which is more costly to set up.

OTHER EXPENSES:

Animal Breeding ($7,500). This amount is needed to maintain breeders of the strains used in this project to generate enough progeny to perform large scale, controlled experiments.

Animal housing ($21,700). This cost reflects the cage charges for all mice immediately before and after infection with ZIKV. All infection experiments will take place in the ABSL3, which has higher per diems. A detailed estimate of the number of mice to be used is provided in the Vertebrate Animals section.

Pharmacy, Surveillance, and Dissection tools ($5,000). This is required for sedation, euthanasia, necropsy, and surveillance for adventitious pathogens within the animal facility

BSL costs ($7,500). This includes additional costs associated with BSL2 and A-BSL3 biosafety laboratory operation (facilities access charges and user fees, personal protective equipment, and BSL3 waste disposal). We have enhanced the safety level of animal studies to A-BSL3 due to added risk of handling infected animals.

Equipment Service Contracts ($6,000). Funds are budgeted for service contracts for laboratory equipment (ELISPOT reader, Miltenyi 8-color flow cytometer, TaqMan ABI 7800, ultracentrifuge).

Other Costs:

Funds are budgeted for publication costs ($2,000) and travel three times per year to a special progress meeting with the members of the CETR ($3,000) as well; as other scientific and Project specific meetings.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		80,033.00
Section B, Other Personnel		468,819.00
Total Number Other Personnel	14	
Total Salary, Wages and Fringe Benefits (A+B)		548,852.00
Section C, Equipment		0.00
Section D, Travel		15,000.00
1. Domestic	15,000.00	
2. Foreign	0.00	
Section E, Participant/Trainee Support Costs		0.00
1. Tuition/Fees/Health Insurance	0.00	
2. Stipends	0.00	
3. Travel	0.00	
4. Subsistence	0.00	
5. Other	0.00	
6. Number of Participants/Trainees	0	
Section F, Other Direct Costs		660,350.00
1. Materials and Supplies	458,500.00	
2. Publication Costs	10,000.00	
3. Consultant Services	0.00	
4. ADP/Computer Services	0.00	
5. Subawards/Consortium/Contractual Costs	0.00	
6. Equipment or Facility Rental/User Fees	0.00	
7. Alterations and Renovations	0.00	
8. Other 1	191,850.00	
9. Other 2	0.00	
10. Other 3	0.00	
Section G, Direct Costs (A thru F)		1,224,202.00
Section H, Indirect Costs		642,707.00
Section I, Total Direct and Indirect Costs (G + H)		1,866,909.00
Section J, Fee		0.00
Section K, Total Direct and Fee (I + J)		1,866,909.00

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

Expiration Date: 03/31/2020

1. Vertebrate Animals Section

Are vertebrate animals euthanized? ● Yes ○ No

If "Yes" to euthanasia

Is the method consistent with American Veterinary Medical Association (AVMA) guidelines?

● Yes ○ No

If "No" to AVMA guidelines, describe method and provide scientific justification

2. *Program Income Section

*Is program income anticipated during the periods for which the grant support is requested?

○ Yes ● No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period *Anticipated Amount ($) *Source(s)

PHS 398 Cover Page Supplement

3. Human Embryonic Stem Cells Section

*Does the proposed project involve human embryonic stem cells? ○ Yes ● No

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, check the box indicating that one from the registry will be used:

❑ Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Cell Line(s) (Example: 0004):

4. Inventions and Patents Section (Renewal applications)

*Inventions and Patents: ○ Yes ● No

If the answer is "Yes" then please answer the following:

*Previously Reported: ○ Yes ○ No

5. Change of Investigator/Change of Institution Section

❑ Change of Project Director/Principal Investigator

Name of former Project Director/Principal Investigator

Prefix:

*First Name:

Middle Name:

*Last Name:

Suffix:

❑ Change of Grantee Institution

*Name of former institution:

Tracking Number: GRANT12598083 Funding Opportunity Number: RFA-AI-17-042 . Received Date: 2018-03-29T16:30:43.000-04:00

PHS 398 Research Plan

OMB Number: 0925-0001
Expiration Date: 03/31/2020

Introduction	
1. Introduction to Application (for Resubmission and Revision applications)	
Research Plan Section	
2. Specific Aims	Flavi_Aims_Final.pdf
3. Research Strategy*	Flavi_Strategy_Final.pdf
4. Progress Report Publication List	
Other Research Plan Section	
5. Vertebrate Animals	Vertebrate_Animals_Flavi.pdf
6. Select Agent Research	
7. Multiple PD/PI Leadership Plan	
8. Consortium/Contractual Arrangements	
9. Letters of Support	
10. Resource Sharing Plan(s)	Flavi_resource_sharing.pdf
11. Authentication of Key Biological and/or Chemical Resources	Authentication_Flavi_Final.pdf
Appendix	
12. Appendix	

Tracking Number: GRANT12598083 Funding Opportunity Number: RFA-AI-17-042. Received Date: 2018-03-29T16:30:43.000-04:00

SPECIFIC AIMS. The flavivirus genus comprises over 70 positive, single-stranded RNA viruses. Many flaviviruses are significant human pathogens, among which dengue virus (DENV) is the most important mosquito-transmitted virus with over 390 million infections per year. West Nile virus (WNV), yellow fever virus (YFV), Japanese encephalitis virus (JEV), tick-borne encephalitis virus (TBEV), and recently Zika virus (ZIKV) also cause global outbreaks and epidemics, posing constant threats to public health[1]. No FDA-approved antiviral drug is available to treat any flavivirus infection, and vaccines are lacking against several family members. Thus, the development of flavivirus antiviral therapy is a public health priority.

The goal of this proposal is to develop direct antiviral agents (DAAs) for flavivirus therapy. Antiviral development for all RNA viruses, including flaviviruses, has two major challenges. **The first challenge is the rapid emergence of resistance** due to the low fidelity of the viral RNA-dependent RNA polymerase (RdRp). Thus, DAAs against different viral targets should be pursued for combination therapy to mitigate resistance, as exemplified by the optimal therapy of hepatitis C virus. During the past funding period of this CETR consortium (AD3C), we identified potent inhibitors of distinct flavivirus protein functions. In the current proposal, we will develop these inhibitors further into clinical candidates through hit-to-lead optimization. These preclinical candidates will form the foundation of combination therapy. **The second challenge is the narrow spectrum of antiviral activity** due to the sequence variations of the binding pockets of DAAs. Nucleoside/nucleotide (Nuc) DAAs can overcome this challenge through targeting the most conserved active sites of viral polymerases. This strategy has been used successfully to combat human viral infections, as Nuc drugs possess broad-spectrum activity and a high resistance barrier when treating HIV and HCV patients[2]. We propose to perform a focused testing of novel Nuc collections from the Emory Institute for Drug Development (EIDD; headed by (b) (3) (A), (b) (6)) and (b) (3) (A), (b) (6) against flaviviruses. In support of this idea, testing of the EIDD Nuc collection against coronaviruses and alphaviruses identified promising inhibitors for **Projects 1 and 2** of AD3C. Based on the above rationale, we propose three Specific Aims to develop flavivirus DAAs (see **Fig 1**).

Aim 1. Develop preclinical DAAs targeting flavivirus capsid and NS4B protein. Previous studies have identified promising inhibitors of DENV capsid (required for flavivirus assembly) and ZIKV NS4B (required for flavivirus replication and immune evasion) proteins. We will perform hit-to-lead optimization to advance these compounds to animal efficacy testing and preclinical development. Specifically, (i) the DENV capsid inhibitor showed *in vivo* efficacy, but low aqueous solubility. We have crystalized DENV-2 capsid protein in complex with the inhibitor, and will perform structure-based rational design to improve its solubility and efficacy. (ii) The ZIKV NS4B compound exhibited <10 nM EC_{50} and >25 μM CC_{50} in cell culture. We will profile the pharmacokinetics (PK) and test the *in vivo* efficacy of the inhibitor in a ZIKV mouse model. For both compound classes, medicinal chemistry will be performed to broaden the antiviral spectrum to other flaviviruses. In addition, we will passage viruses in the presence of drug candidates to define the potential for resistance, followed by characterization of the resistant viruses for their replication fitness *in vitro* and pathogenic potential *in vivo*.

Aim 2. Identify novel Nuc DAAs with pan-flavivirus activity. Using DENV as a pilot, we will identify pan-flavivirus Nuc inhibitors from the EIDD and (b) (3) (A), (b) compound collections. We will test concurrently (i) a nucleoside (that require host kinases to convert to nucleoside triphosphate for antiviral activity) collection in a cell-based DENV infection assay and (ii) a nucleoside triphosphate collection in a biochemical RdRp assay. Compounds with activities in both assays will be prioritized for development because they are efficiently converted to nucleoside triphosphate by host kinases. Compounds without activity in (i), but with activity in (ii) are not efficiently converted to nucleoside triphosphate by host kinases; we will pursue phosphonate or phosphoramidate prodrugs to rescue these Nucs for development. For identified Nucs, we will characterize their spectrum against other flaviviruses, confirm mechanism of action, assess *in vivo* protection, and evaluate resistance.

Aim 3. Evaluate combination therapy directed against flavivirus infection. Combination therapy is essential to prevent resistance during antiviral treatment. We will test therapeutic regimens combining DAAs with distinct viral targets from Aims 1 and 2. We will first examine the synergistic or additive effects of DAAs in cell culture. The most effective combinations from cell culture will be evaluated in mouse pathogenesis models. Finally, the most promising DAA combination will be validated in non-human primate (NHP) models of flavivirus infection and will be used to guide future clinical implementation.

We will deliver the proposed milestones by combining the cutting-edge biology from academia and drug discovery acumen from industry along with defined **Go/No-Go** decision algorithms and with input from the Scientific Advisory Committee (SAC). The collective effort addresses a major unmet medical need by bridging academia and industry, including investigators from University of Texas Medical Branch (Pei-Yong Shi), Washington University in St. Louis (b) (3) (A), (b) (6)), Oregon Health Science University (b) (3) (A), (b) (6), EIDD ((b) (3) (A), (b) (6)), Southern Research (Ashish Pathak), and (b) (3) (A), (b) (6)

SIGNIFICANCE. Although many flaviviruses cause frequent global epidemics/outbreaks, no clinically approved therapy is available to treat these viral infections. This project aims to develop preclinical candidates for flavivirus therapy. To achieve this goal, we will focus our effort first on two globally relevant flaviviruses with different disease pathogenesis and associated clinical syndromes. (i) **DENV** causes a febrile syndrome, which can progress to vascular leakage, shock syndrome, and hemorrhagic manifestations in severe cases. (ii) **ZIKV** causes devastating congenital disease in fetuses from infected pregnant women and is associated with Guillain-Barré syndrome in adults. Once inhibitors are identified, we will expand the antiviral potential to other medically important flaviviruses, including YFV, WNV, JEV, and TBEV. By reciprocally testing compounds from different project teams of this CETR consortium, we also will evaluate whether flavivirus inhibitors have broad spectrum activity against coronaviruses (**Project 1**), alphaviruses (**Project 2**), and/or influenza virus (**Project 4**).

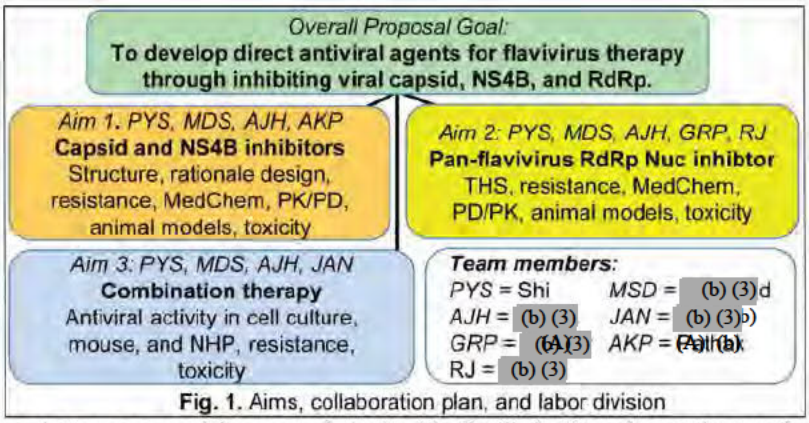


Fig. 1. Aims, collaboration plan, and labor division

DENV threatens >3.6 billion people and causes 390 million human infections annually, of which ~96 million infections manifest symptoms[3]. DENV infection leads to dengue fever (DF), life-threatening hemorrhagic fever (DHF), or shock syndrome (DSS). DENV is endemic in much of the tropical and subtropical regions. The rise in DENV cases and expanded geographical distribution over the past three decades is due to increased urbanization of the human population and spread of *Aedes* mosquitoes, the primary transmission vector. Additionally, international travel, military campaigns, and global shipping have transported the virus and its mosquito vector to new regions[4]. There are four serotypes of DENV with approximately 30-35% amino acid variation among the structural and non-structural proteins[5]. Because of this genetic variation, the development of a tetravalent DENV vaccine has proven challenging. An effective DENV vaccine needs to induce long-lasting immune protection against all four serotypes; a vaccine recipient with incomplete immunization may be sensitized to severe DHF or DSS upon subsequent DENV infections. This challenge was reflected by the results of three phase 2b/3 trials of Dengvaxia®: an average efficacy of 30-61%[6-9]. Unfortunately, Dengvaxia® also seems to increase the risk of hospitalization over time in children <9 years of age who are seronegative at the time of vaccination, possibly through vaccine-induced antibody enhancement. The WHO recently recommended the use of Dengvaxia® only in areas where the disease is highly prevalent[10]. Given the challenges with DENV vaccines, antiviral therapy must be pursued as an alternative mechanism to control and treat DENV infection.

ZIKV has emerged explosively as a major public health threat. Like DENV, ZIKV is primarily transmitted by *Aedes* species mosquitoes. In addition, ZIKV can infect humans through a sexual route, blood transfusion, organ transplantation, and maternal-fetal transmission[11-20]. ZIKV initially was isolated from a sentinel rhesus macaque in 1947 in the Zika Forest of Uganda[21]. Until 2007, ZIKV had circulated silently in many parts of Africa and Asia without causing severe disease or large outbreaks, with fewer than 20 documented human infections[22]. During the recent epidemics, ZIKV infection has caused devastatingly severe disease, including congenital malformations in the fetus of infected pregnant women (*e.g.*, microcephaly and fetal demise) and Guillain-Barré syndrome in adults[23-25]. Phylogenetic analysis revealed that ZIKV evolved into African and Asian lineages[26]. Strains from the Asian lineage are responsible for the recent large-scale epidemics on the Yap Island in 2007, in the French Polynesia and South Pacific in 2013, and in the Americas in 2015 and 2016[11,27]. In the past three years, ZIKV vaccine development has progressed rapidly, including inactivated, subunit, and live-attenuated vaccines[28-37]. Several ZIKV vaccine candidates have entered or completed phase I clinical trials[33,38]. Compared with vaccine development, progress on ZIKV therapeutics has lagged. Although a number of laboratories have identified small molecular inhibitors (primarily through screening FDA-approved drugs) and therapeutic antibodies, none has entered clinical trials[39].

Flaviviruses have a positive-sense single-stranded RNA genome of approximately 11,000 nucleotides. The genome contains a 5' untranslated region (UTR), single open-reading frame (ORF), and 3' UTR. The ORF encodes three structural (capsid [C], precursor membrane [prM], and envelope [E]) and seven non-structural (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) proteins. The structural proteins form virus particles and function in viral entry into cells. The nonstructural proteins participate in viral replication, virion assembly, and evasion of host innate immune responses[40]. In the past fifteen years, significant progress has been made in understanding the structural biology of flavivirus replication. This knowledge (*e.g.*, the atomic structures of all viral enzymes and structural proteins) has enabled rational drug discovery. Two general strategies have been pursued for flavivirus antiviral discovery: **Strategy I** is to develop *bona fide* flavivirus inhibitors; **Strategy II** is to

repurpose existing clinical compounds and drugs approved for other clinical indications. For the latter strategy, five clinically developed compounds have been tested in dengue patient trials: chloroquine, a malaria drug that has anti-fusion and anti-virion maturation activities as well as immunomodulatory activities[41]; prednisolone, a corticosteroid drug that suppresses inflammation[42]; balapiravir, a cytidine analog that inhibits the viral RNA dependent RNA polymerase (RdRp)[43], celgosivir, an inhibitor of host α-glucosidase that modifies viral E and NS1 proteins[44]; and lovastatin, a cholesterol synthesis inhibitor[45]. None of these repurposed drugs showed any significant antiviral effect or clinical benefit in dengue patients. Although repurposing of FDA-approved drugs is an attractive approach to expand rapidly the use of available medicines to new indications, two key criteria must be met for development. First, the exposure level of the drug in humans should exceed the concentration of efficacy determined in cell culture (*e.g.*, EC_{90} values). Second, since different diseases are often associated with infection of different tissues or organs, it is important to focus on drug exposure levels in the disease-related tissues. In contrast to the repurposing strategy, there has been no *bona fide* compound specifically designed for flavivirus therapy that has entered clinical trials. We believe it is critical to develop *bona fide* flavivirus inhibitors.

INNOVATION. The innovation of this project derives from the clear research plan for identifying three types of DAAs. **For the DENV capsid (C) inhibitor**, we have defined the limitation of an existing inhibitor: poor aqueous solubility. Using a newly obtained co-crystal structure of compound-capsid complex, we will rationally design analogs to improve the compound solubility, potency, and antiviral spectrum. Since the current model compound has demonstrated *in vivo* efficacy, we expect to achieve compounds with improved physicochemical property that maintain antiviral efficacy in DENV animal models. **For the ZIKV NS4B inhibitor**, the current frontrunners have a cellular efficacy of **EC_{50} <10 nM** and CC_{50} >25 μM. PK profiling and *in vivo* efficacy testing are the next steps for generation of a clinical candidate. Since NS4B does not contain any enzymatic activity, targeting of this viral protein represents a novel approach for flavivirus drug discovery. **For the Nuc inhibitor**, we will take a systematic approach to test Nuc compounds from EIDD and (b) (3) (A), (b) that have never been tested for flaviviruses. Testing of the EIDD compounds has already has generated promising inhibitors of coronaviruses (**Project 1**) and alphaviruses (**Project 2**). The proposed testing effort is further justified, as Nuc inhibitors represent the cornerstone of the current antiviral therapy in patients. The three antiviral approaches of this project are well-delineated, justified, and expected to deliver promising pre-clinical drug candidates.

SCIENTIFIC PREMISE. The project goal is to develop DAAs for flavivirus therapy. Drug resistance and antiviral spectrum are two challenges for anti-infective development. We will take a two-pronged approach to overcome the challenges: (i) Combination therapy with inhibitors of distinct viral targets (*i.e.*, capsid, NS4B, and RdRp proteins) to prevent resistance; (ii) Nuc inhibitor targeting the most conserved active site of viral RdRp to achieve pan-flavivirus activity. We have defined a clear plan of action with **Go/No-Go** criteria to address these issues and ensure the delivery of the proposed milestones.

SCIENTIFIC RIGOR. (i) Power calculations for animal use. Experiments in this proposal will use mice and NHP to identify compounds with *in vivo* antiviral potential. Prospective sample size estimations using power calculations will be performed for mice (see **Vertebrate Animals** for details). Mouse experiments will be performed in small cohorts with controls (*e.g.*, vehicle or mock-inoculation) and repeated several independent times to ensure reproducibility. **(ii) Reducing bias.** Mice will be randomized into experimental groups, and researchers performing the analysis will be blinded to the experimental groups. **(iii) Improving precision and reducing variability.** All experiments will include multiple biological and technical replicates, and negative and positive (*e.g.*, NITD008) controls. Results will be analyzed by parametric or non-parametric analyses (depending on data distribution) with corrections for multiple comparisons. Reporting of experimental data will be transparent (*e.g.*, scatterplot or uploading of data) so others may reproduce, analyze, or extend findings.

RELEVANT BIOLOGICAL VARIABLES. Viruses, mice, and NHP are the biological systems used in this proposal. Virus stocks will be generated from infectious clones and sequence-confirmed prior to use for infection studies. AG129, hSTAT2-KI, and C57BL/6J wild-type (WT) mice will be bred in our animal facilities and purchased from Jackson Laboratories. SNP analysis will be performed to confirm congenic status, and further backcrossing will be performed, if necessary. Genotyping of all mice will be performed to confirm expression of key genes. Conventional animal housing conditions will be used, as there is no evidence that altered room temperatures and light/dark cycles influence flavivirus pathogenesis. Mice and NHP of both sexes will be used, and phenotypes will be monitored for possible sex-based differences.

COLLABORATION PLAN AND DIVISION OF LABOR. As outlined in **Fig. 1,** a multidisciplinary team with drug discovery acumen and a track-record in flavivirus biology and animal studies will work together to deliver milestones. The **Shi lab** will perform DENV capsid/inhibitor structural study, ZIKV NS4B inhibitor analysis, and Nuc characterization. The (b) (3) (A), (b) (6) **lab** will perform ZIKV and WNV mouse studies, antiviral spectrum analysis, and host mechanism analysis. The (b) (3) (A), (b) (6) **labs** will pursue ZIKV 'hits' from the current CETR

screening and perform NHP efficacy studies. The **Pathak group** will design and execute non-Nuc MedChem as well as all PK analysis (for both non-Nuc and Nuc). The (b) (3) (A), (b) (6) will provide Nuc compounds and synthesize Nuc pro-drugs. (b) (3) (A), (b) (6) will provide Nuc compounds and input on **Go/No-Go** decisions.

BACKGROUND AND PRELIMINARY DATA

(b) (4)

DENV capsid (C) inhibitor. From a DENV infection screening, Byrd et al[46] identified an inhibitor ST-148 (**Fig. 2A**) targeting the C protein. This compound inhibited DENV-2 in a viral titer reduction assay with an EC_{50} of 16 nM and an EC_{90} of 125 nM. However, it displayed weaker activity against other serotypes, with EC_{50} values of 2.8, 0.5, and 1.2 μM for DENV-1, DENV-3, and DENV-4, respectively. The resistance was mapped to a single amino acid change of S34L in the DENV-2 C protein. Infectious virus with the engineered mutation showed a 550-fold reduction of susceptibility to ST-148. Twice daily treatment with ST-148 reduced peak plasma viremia by 52-fold and reduced viral load in the spleen and liver by 3- and 20-fold, respectively. Mechanistically, ST-148 was proposed to enhance C protein self-interaction and induce structural rigidity, leading to perturbation of nucleocapsid assembly and disassembly[47]. As part of our current CETR project, we identified a structurally similar compound SRI-40384 (**Fig. 2A**) that inhibits DENV-2 with an EC_{90} of 40 nM, but also is less effective against other DENV serotypes. We found that SRI-40384 incorporates into nascent virions, which are less infectious than virions from untreated cells, consistent with the hypothesis that C disassembly is impeded by the drug. Furthermore, we identified multiple mutations conferring resistance to SRI-40384 (*e.g.*, L35P and L38M) that localize to residues proximal to S34, suggesting that these compounds target the same region of C. Based on the complementary data (from our current CETR and SIGA Technology), we propose to take a "fast follow-up" approach to further develop this compound series.

A major limitation of the ST-148 series is low aqueous solubility (<10 μM). To facilitate hit-to-lead optimization, we recently solved the co-crystal structure of DENV-2 C protein in complex with ST-148. The complex crystallized in the space group *P*2 with one dimer in the asymmetric unit. The structure was solved by molecular replacement using the free WNV C structure[48] to 1.25 Å resolution. As shown in **Fig. 2B**, two molecules of ST-148 form a head-to-tail conformation. The compounds bind to a pocket that is formed at the interface between two C dimers. The pocket is formed by helices α-1 to α-3 of the dimeric C proteins. The seven-membered ring of ST-148 sits deep into the pocket created in each dimer and ensures that the compound can fit only in a head-to-tail conformation. The co-crystal structure supports the mechanism that ST-148 interferes with the oligomerization of C dimers, leading to the perturbation of nucleocapsid assembly and disassembly[47]. The structural information has provided the basis for rational design of ST-148 analogs with improved solubility and potency. Comparison of the ST-148 binding pocket in DENV-2 C with the analogous regions of C from ZIKV and WNV[48,49], which are highly conserved in sequence and structure, will allow us to design analogs with a broader spectrum of activity.

ZIKV NS4B inhibitor. We also will pursue potent inhibitors of ZIKV that were identified by screening compound libraries from (b) (3) (A), (b) and Southern Research (SR). Screening of the (b) (3) (A), (b) library identified several structurally similar hits with EC_{50} of 0.5-2 μM and CC_{50} of >25 μM. The medicinal chemistry effort improved the potency of this scaffold (GS-1 and GS-2) to EC_{50} <10 nM and CC_{50} >25 μM (**Fig. 3A**). Antiviral spectrum testing showed the compounds are less active against DENV, YFV, and other flaviviruses with EC_{50} >1 μM, suggesting that the compounds selectively inhibit ZIKV.

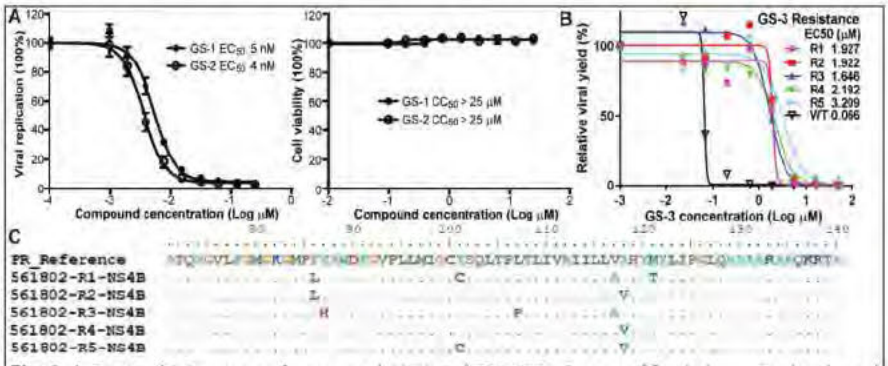


Fig. 3. A. EC_{50} and CC_{50} curves of compounds GS-1 and GS-2. B. Resistance of five independently selected ZIKVs (R1 to R5). The EC_{50} values of the WT and five resistant viruses are indicated. C. Amino acid mutations in the NS4B gene. ZIKV PRVABC59 is used as a reference. Amino acid positions of NS4B are indicated.

To de-convolute the target of the (b) (3) (A), (b) inhibitors, we selected for resistant viruses by culturing an epidemic ZIKV strain (PRVABC59) on Huh7 cells with increasing concentrations of GS-3, an analog to GS-1 and GS-2, as done previously[50,51]. Viruses derived under GS-3 selection were subjected to antiviral testing against GS-3 using a quantitative RT-PCR method. **Fig. 3B** shows the antiviral curves of five independently selected resistant viruses. The EC_{50} value increased from 0.07

μM for the WT virus to 1.6-3.2 μM for the selected viruses, a 25- to 48-fold increase. Full-genome sequencing of the five resistant viruses revealed mutations in NS4B (**Fig. 3C**). The results suggest that GS-3 likely targets the ZIKV NS4B protein.

In addition, compounds with anti-ZIKV activity were identified through a supplemental screen of SR libraries. We selected three inhibitors of ZIKV with EC_{90} values <1 μM and favorable structural properties for MedChem effort (**Table 1**). These compounds inhibited ZIKV by 2-3 logs at non-toxic concentrations, among which SRI-35814 also inhibited DENV-2 (EC_{90} 2.6 μM) and WNV (EC_{90} 1 μM). SRI-35804 and SRI-35814 are structurally similar, but possess poor solubility (<10 μM). An iterative design-synthesis-testing process will be used to identify novel and potent analogs with drug-like (*i.e.*, physicochemical and pharmacokinetic) properties and to develop a structure-activity relationship (SAR) within a chemical series. We will pursue these compounds in parallel with the (b) (3) (A) (b) scaffold to expand and diversify the discovery pipeline.

(b) (4)

Two types of testing for Nuc compounds. For any Nuc compound, it is important to determine (i) if the Nuc triphosphate (NTP) can inhibit viral RdRp in a biochemical enzyme assay and (ii) if the Nuc is converted efficiently to its triphosphate form inside cells. These two parameters determine the therapeutic potential and the developmental approach for each Nuc. We have established assays to measure these two parameters. A **fluorescence-based alkaline phosphatase-coupled polymerase assay** (FAPA) has been developed to measure the IC_{50} of NTP (**Fig. 4**). A modified nucleotide analogue (2'-[2-benzothiazoyl]-6'-hydroxybenzothiazole) conjugated adenosine triphosphate (BBT-ATP) and a viral RNA were used as substrates in a DENV RdRp assay. After the polymerase reaction, treatment with alkaline phosphatase liberates the BBT fluorophore from the polymerase reaction by-product BBT(PPi), which can be detected at excitation and emission wavelengths of 422 and 566 nm, respectively. This assay is reliable for testing DENV RdRp inhibitors with a Z' factor of 0.81, as reported previously[52]. To gauge the efficiency of Nuc triphosphate conversion, we developed **a luciferase DENV-2 infection assay** (Z' factor of 0.79) that provides a robust screen for inhibitors in cell culture[53]. Nuc compounds with antiviral activities in the luciferase DENV-2 infection assay suggest that they are converted to the triphosphate form by the host kinases. Testing Nuc compounds using these two assays will identify Nuc inhibitors and help define the path to development (see **Aim 2**).

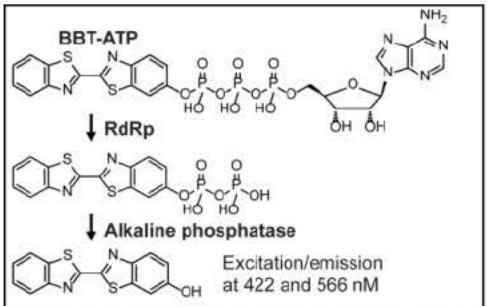


Fig. 4. FAPA assay. Chemical structures of BBT-ATP and its metabolites during the assay are shown.

APPROACH

Overall rationale. Four general approaches could be pursued for antiviral therapy[39]. ***(i) Inhibition of viral targets***. The majority of current antiviral drugs in clinical use are inhibitors of viral enzymatic activities, such as HIV reverse transcriptase, protease, and integrase. ***(ii) Inhibition of host factors*** required for viral infection. This approach has led to the development of Maraviroc, a clinically approved HIV drug that inhibits the host protein CCR5, a co-receptor for HIV entry[54]. ***(iii) Immune modulators***. Interferon, a potent cytokine that triggers protective defenses of immune system to eradicate pathogens, has been used clinically for treatment of HCV and other viral infections[55,56]. ***(iv) Inhibition of disease pathway***. Since disease pathology can be caused by an over-exuberant immune response to viral infection, molecules that modulate immune pathways may mitigate disease. This approach is attractive for treatment of acute viral infections because these infections often exhibit a relatively short duration of viral replication that declines to undetectable level within a few days. However, such approaches require a clearly defined molecular mechanism of the disease pathway, which currently is missing for many viral infections. Since approach (i) has contributed to almost all antiviral drugs currently in clinical use, we have opted to follow this proven approach to develop DAAs for flaviviruses in Project 3. Moreover, clinical studies with DENV have shown that the levels of viral replication and viral proteins is linked to disease severity[57,58]. Thus, DAAs that reduce viral burden should improve clinical outcome. Specifically, we propose to pursue three well-defined viral targets and their potential inhibitors for preclinical development.

Aim 1. Develop preclinical DAAs targeting flavivirus capsid and NS4B proteins.

Rationale. The rapid emergence of resistance has been a challenge for antiviral therapy. Combination antiviral therapy is a solution to prevent resistance in patients. Therefore, DAAs against different viral targets will be pursued as combination therapy. In this Aim, we propose to perform hit-to-lead optimizations of two potent inhibitors: a DENV capsid inhibitor and a ZIKV NS4B inhibitor. **Rationale for DENV capsid inhibitor:** During the past fifteen years, inhibitors of distinct viral proteins have been reported for different flaviviruses. Unfortunately, direct flavivirus inhibitors have shown limited efficacy in animal models[5]. Therefore, a starting compound with an established *in vivo* efficacy represents an advantage. Based on this rationale, we have chosen to perform hit-to-lead optimization of a DENV C inhibitor, which has antiviral activity in a mouse model[46]. The co-crystal structure of compound-C protein complex will enable us to rationally design analogs. **Rationale for ZIKV NS4B inhibitor:** Flavivirus NS4B inhibitors are among the most frequent hits of viral targets when performing cell-based infection screens[59]. Indeed, DENV and YFV NS4B inhibitors have shown efficacy in animal models[51,60]. Based on this rationale, we propose to develop further a potent ZIKV NS4B inhibitor identified from (b) (3) (A). focused library. Additionally, we will pursue development of several compounds with anti-ZIKV activity identified by AD3C; these hits will be optimized for potency, drug-like properties, and characterized for mode of action through resistance analysis.

(b) (4)

1.1. Improvement of a DENV capsid inhibitor. Although ST-148 has been shown to have antiviral activity in the AG129 mouse model of DENV pathogenesis, it has poor solubility (<10 μM) and high lipophilicity (LogD >4.5). We will take two complementary approaches to improve the drug-like properties of ST-148. **First, we will explore systematic SAR of ST-148** through medicinal chemistry in which analogs will be synthesized to increase solubility while retaining potency and microsomal stability. One obvious structural alteration is to remove the 7-membered aliphatic ring system and introduce substituents at the pyridine ring to increase solubility. It has been seen that this change retains the activity, as exemplified by UTMB-001 (**Fig. 5**). Some areas of chemical modifications are shown in **Fig. 5** in which a few sets of compounds at each indicated site will be designed and synthesized. Newly synthesized compounds will follow the work-flow (see **Fig. 7**). Compounds will be tested for their activity, potency, and cytotoxicity to generate SAR data. The design of new compounds will follow the Lipinski's Rule of Five, as well as based on calculated drug-like properties of new analogs, such as solubility (LogS), lipophilicity (clogP), and StarDrop™ Composite Site Liability (CSL) for metabolically unstable areas in the molecule. These criteria will be used as a guide for designing and prioritizing targets. The calculated CSL values are used as a measure of the metabolism efficiency of a molecule by six of the major drug metabolizing isoforms of CYP450. Solubility, LogD, and microsomal stability also will be determined experimentally on compounds to guide the design and synthesis of analogs with better PK properties. **Second, we will use the co-crystal structure of ST-148-C protein to design compounds** with improved solubility and potency. As shown in **Fig. 6A**, two ST-148 molecules bind to a pocket formed at the interface of two C dimers; there is extra space in the pocket that could be occupied by extending the ST-148 molecule. To explore this hypothesis, we will synthesize "extended" compounds to fully occupy this pocket, with UTMB-002 being one such compound (**Fig. 6B**). Active compounds from both approaches will be co-crystallized; the new co-crystal structures will guide iterative compound design.

(b) (4)

The Medicinal Chemistry and Lead Development Core (MCLDC; Core-C) team will coordinate all *in vivo* PK studies of selected compounds using well established protocols at Pharmaron Inc. Compounds will be prioritized and selected for *in vivo* PK studies in mice based on activity, potency, and *in vitro* ADME (EC_{90} < 1 μM; CC_{50} > 30 μM; VTR > 3 logs at 10 μM; MLM $t_{½}$ >60 min; Sol. >10 μM). These PK studies preferably will be performed via oral (PO) administration of the compound, but if acceptable exposure of a compound is not obtained via oral administration (bioavailability F >30%, half-life >2h), other routes of administration [intravenous (IV), subcutaneous (SC) or intraperitoneal (IP)] will be investigated. All of these properties will serve as guidelines to determine *in vivo* candidates. The PK profile of a compound will be assessed in the AG129 mice strain following single PO administration (*e.g.*, dose: 5 mg/kg, vehicle: 2% DMSO/3% solutol in water). Properties, such as plasma half-life ($t_{1/2}$), peak plasma concentration (C_{max}), time to reach C_{max} (T_{max}), systemically available fraction of a compound (bioavailability %F), clearance (CL), volume of distribute (V_d), and area under the curve (AUC_{inf} and AUC_{last}) will be determined. Compound dose and frequency of administration for *in vivo* efficacy studies in murine models will

be calculated based on these PK properties. If acceptable exposure of compound is not achieved by the above referenced formulation, other formulations will be used to improve PK properties. Compounds that are efficacious in animals should show at least a three-fold greater compound concentration in plasma than the measured EC_{90}.

It will be important to understand metabolic profile and off-target interactions of compounds with acceptable PK profiles and *in vivo* efficacy. For example, assessment of the potential of a compound to inhibit specific cytochrome P450 enzymes, the major enzymes associated with metabolism in the liver. This interaction may affect plasma levels *in vivo* and potentially could lead to adverse drug reactions or toxicity. Specifically, the six major drug metabolizing CYP450s in humans will be tested: CYP1A2, CYP3A, CYP2D6, CYP2C9, CYP2C19, and CYP2E1. In addition, advanced compounds also will be tested in a panel of assays for off-target liabilities. This will be done by **Core C** through testing in a wide range of target classes including GPCRs, kinases, and nuclear receptors at 10 μM. Any activity <50% inhibition will be evaluated further. In addition, compounds also will be tested in the mini-Ames mutagenicity test [mutagenic potential of new drugs in two strains of *Salmonella typhimurium* (TA98 and TA100)] and evaluated for hERG activity (the binding and inhibition of the hERG potassium channel provides critical information on the potential of the compound to cause cardiac risk; Low: IC_{50} > 10 μM; Moderate: 1 μM < IC_{50} < 10 μM; High: IC_{50} < 1 μM).

1.2. Characterization of DENV C inhibitors. Resistance analysis will confirm the on-target antiviral activity of the new analogs. We will perform two types of resistance experiments. The first experiment will test if the new compounds are cross-resistant to DENV-2 S34L mutant that was previously selected from ST-148[46]. Cross-resistance will suggest that the new analogs are on-target to inhibit C protein. We expect most of the analogs will be cross-resistant to the S34L virus. However, some may have subtle differences in binding to the C protein, leading to decreased resistance to the S34L mutant virus. If this is the case, we will initiate new resistance selection against these compounds[46]. Complete genome sequencing of the selected viruses will reveal if resistant mutations map to the C gene. Using an existing infectious clone[61], recombinant viruses containing these mutations (single or in combination) will be prepared to validate their roles in resistance. In addition, new compounds with on-target inhibition will be co-crystallized with the C protein for confirmation of on-target activity. The co-crystal structure information will be used to guide iterative compound design and synthesis.

We will monitor the antiviral spectrum of new analogs. During the medicinal chemistry efforts, new compounds will be routinely tested against four serotypes of DENV. Conserved sequence and structure of the C proteins from DENV-1 to DENV-4, WNV, and ZIKV[48,49], together with the location of resistant mutation(s) on the co-crystal structure of compound-C protein complex, will facilitate the design of pan-DENV and possibly pan-flavivirus C inhibitors. We will test the new compounds against ZIKV, WNV, YFV, JEV, and TBEV using viral titer reduction assays. These experiments should lead to compounds with broader antiviral spectrums.

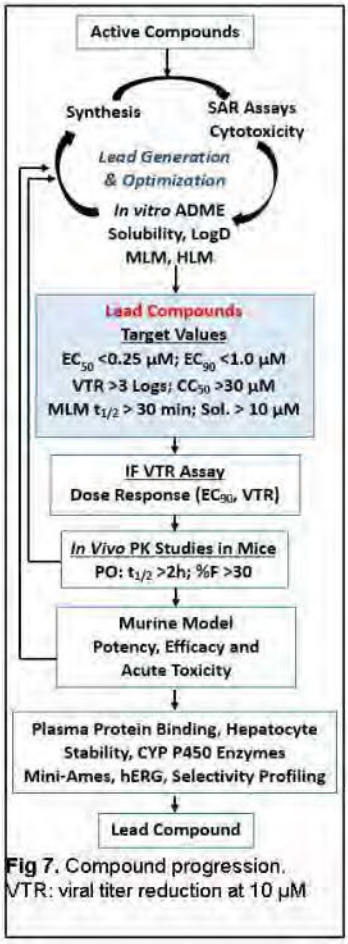


Fig 7. Compound progression. VTR: viral titer reduction at 10 μM

Advanced compounds with favorable PK profiles will be tested for their *in vivo* efficacy in the AG129 (lacking IFN-α/β and IFN-γ receptors) mouse model of DENV pathogenesis. AG129 mice will be housed at UTMB in an A-BSL2 animal facility. Groups of 10 to 15 8-week-old mice will be inoculated for each condition, and equal numbers of negative and positive controls will be included. For experiments designed to appreciate smaller effects on efficacy parameters, greater numbers of mice will be used to increase the statistical power of the study. Each mouse will be inoculated with 4×10^6 PFU of DENV-2 strain TSV01 via the IP route. Initially, we will test the compound efficacy by starting the treatment within 15 min of viral infection, with the specific dosing regimen and route of administration determined by their PK profiles. If efficacy is achieved, we will pursue post-exposure efficacy by delaying the start of treatment at 12, 24, 36, and 48 h post-infection. We will measure four parameters to indicate compound efficacy: (i) Viremia at days 3 and 4 post-infection using plaque assay on BHK-21 cells[62]; (ii) inflammatory cytokine and chemokine levels of TNF-α, IL-6, IFN-γ, MCP-1, and IL-12p70; (iii) body weight loss; and (iv) survival[63].

Using the AG129 mouse model, we will evaluate the viral fitness and pathogenic potential of resistant DENV by comparing their replication kinetics to the WT virus. In addition, we will examine the vector fitness of the resistant viruses in *Aedes aegypti* mosquitoes through blood meal feeding; this may predict whether a drug-resistant mutant will be maintained in the enzootic cycle. Human red blood cells (Lampire Biological Laboratory) spiked with an appropriate concentration of

resistant viruses (5×10^4 to 5×10^6 PFU/ml; depending on their replication titers from cell culture) will be fed to mosquitoes. Engorged mosquitoes will be analyzed for infectious viruses on day 14[64]. These results will estimate the mosquito infectivity of the resistant viruses.

1.3. ZIKV NS4B inhibitors. Since medicinal chemistry has already improved the potency of the ZIKV NS4B inhibitors to single-digit nanomolar EC_{50} values, this compound will enter the workflow (**Fig. 7**) beginning with ADME and PK profiling. The *in vivo* PK studies will be performed by MCLDC and follow guidelines described in section **1.1**. These properties will serve as guidelines to determine *in vivo* candidates. Compounds that are efficacious in animals should show at least a three-fold greater concentration in plasma than the measured EC_{90}.

We will test the *in vivo* efficacy of compounds using a newly developed immunocompetent human STAT2-KI (hSTAT2-KI) mouse model of ZIKV infection and pathogenesis[65]. The hSTAT2-KI mice will be housed at Washington University. Groups of 10 to 15 3-week-old mice will be infected with 10^5 PFU of a Brazilian strain of ZIKV (Paraiba 2015) via the subcutaneous route. We will first test the compound efficacy by starting the treatment immediately after infection. If efficacy is achieved, we will pursue post-exposure efficacy by delaying the start of treatment at 12 or 24 h post-infection. Three parameters will be measured to indicate compound efficacy: (i) peak viremia at day 2-3 post-infection using plaque assay on Vero cells; (ii) body weight loss; and (iii) viral RNA levels in peripheral (*e.g.*, spleen, testis, eye, vagina) and CNS organs at day 9 after infection. For inhibitors with promising therapeutic profiles, additional kinetic analyses will be performed to determine the effects of an inhibitor on viral pathology and burden. Tissue samples from several organs (*e.g.*, brain, spleen, lymph nodes, kidneys, and testis) will be collected at necropsy. Organs will be evaluated for viral infection, tissue injury, and immune system response by approaches that are established in our laboratories.

Once efficacy has been achieved in non-pregnant mice, we will test the compound's efficacy in a newly-developed immunocompetent mouse model of ZIKV infection in pregnancy. **We acknowledge that evaluation and ultimate use of antiviral drugs in pregnancy is a complex issue that requires extensive safety testing.** Notwithstanding this point, antiviral drugs (*e.g.*, against HIV) are used in pregnant women to prevent congenital transmission. Thus, it is important to test in pregnancy our lead 'hits' that have established efficacy against ZIKV in non-pregnant mice. To do this, 11-week-old hSTAT2-KI pregnant mice (mated to hSTAT2-KI sires) will be inoculated with 10^5 PFU of ZIKV strain Dakar 41525 (which is more pathogenic in the pregnancy model) at embryotic day E6.5 via the SC route. The infected mice will be treated with compound starting at day E7, E8, and E9 post-infection. On day E13.5, the pregnant mice will be measured for (i) viral load in maternal blood, spleen, brain, and in the placenta, and fetal brain; (ii) fetal body weights; and (iii) number of viable fetuses. For the most promising compounds, we will allow dams to deliver pups, and measure the antiviral effects on the number and phenotype of live pups. The pregnant mouse model was established in our laboratories for Zika vaccine projects[36,37]. Although use of a ZIKV therapeutic may be most beneficial for treatment of ZIKV-infected pregnant women, extensive analysis of potential drug teratogenicity itself must be conducted. Although such testing is beyond the scope of this proposal, we acknowledge that additional teratogenicity analysis must be carried out if these experimental compounds prove effective in these studies.

Resistance selection has identified mutations in the ZIKV NS4B gene (**Fig. 3**). To validate their contribution to resistance, using our infectious virus clone[64], we will generate recombinant ZIKV containing these mutations (singular or in combination) and examine their drug sensitivities using plaque assays. For resistant viruses, we also will analyze their replication in cell culture (Vero cells from monkey and defective in type-I IFN production, A549 cells from human lung and competent in type-I IFN production, and C6/36 cells from *Aedes* mosquito), replication fitness, pathogenic potential in hSTAT2-KI mice, and mosquito infectivity.

Since flavivirus NS4B functions as an essential replication component, inhibitors could function by interfering with protein-protein interactions during replication complex formation. DENV NS4B was shown to oligomerize itself[66] as well as to interact with NS1[67], NS3[68,69], and NS4A[70]. Besides binding to viral proteins, NS4B also may interact with host factors (*e.g.* autophagy, type I IFN signaling) during viral replication[59,71-73]. Small molecule inhibitors blocking such interactions could lead to suppression of viral replication. To test these hypotheses, we will perform immunoprecipitation experiments to examine if the NS4B inhibitors block the above mentioned protein-protein interactions as we have previously done[74]. We will perform two sets of experiments. (i) Huh7 cells propagating ZIKV subgenomic replicons[75] will be treated with the inhibitor at its EC_{90} concentration. At 10 h post-treatment, cell lysates will be analyzed by immunoprecipitation using antibodies to specific pairs of proteins (*e.g.,* NS1/NS4B, NS3/NS4B, NS4A/NS4B, or NS4B oligomerization). (ii) Individual pairs of proteins of interest will be transiently expressed in HEK-293 cells using pXJ plasmid system[76]. At 24 h post-transfection, the cells will be treated with the inhibitor at its EC_{90} concentration. At 36-72 h post-transfection, cell lysates will be analyzed by immunoprecipitation. These two approaches will allow us interrogate viral protein-protein interactions in the presence (i) and absence (ii) of viral replication. If differences in viral protein-protein interactions are not observed, we will consider examining changes in NS4B interactions

with host proteins as well. We have extensive experience in DENV NS4B biology and its inhibitors[50,51,59,66,70], which can be applied here.

1.4 Analysis of additional novel ZIKV inhibitors. In addition to the ZIKV NS4B inhibitor, three compounds with potent anti-ZIKV activity will be pursued in parallel. Targets of these compounds are currently unknown. Development of these compounds will follow that of GS-1 by ~1 year, given that the (b) (3) (A) (b) compounds already have undergone extensive SAR to optimize efficacy. Because these compounds are still classified as early leads, **Core C** will synthesize analogs to identify SAR of antiviral activity as shown in **Fig 7** and a similar approach as described for DENV series in Section **1.1**. Viral replication assays using immunofluorescent detection of viral proteins and quantification of viral titers in cultured cells, as well as PK/toxicology evaluations (as described above) at SR will be employed in an iterative design-synthesis and testing process to improve compound potency, selectivity, and drug-like properties (solubility and microsomal stability).

Expected outcomes and alternative approaches. We expect to deliver preclinical inhibitors of DENV C and ZIKV NS4B proteins. The modified DENV C inhibitor will have better solubility (>50 μM) and other physicochemical properties, leading to improved PK and amenable formulation. The co-crystal structure of compound-protein complex will facilitate rational design of analogs with potential pan-DENV and pan-flavivirus activities. For the ZIKV NS4B project, the current front-runners already have reached single-digit EC_{50} potency. The proposed PK/PD study should allow us to achieve *in vivo* efficacy in mice. As demonstrated by our medicinal chemistry capability to refine efficacy to nanomolar potency, we will further improve the ZIKV inhibitors through chemistry efforts if any PK parameter(s) need optimization. Optimization for one parameter may negatively impact another parameter, and multiple modifications may be needed to achieve the desired potency and preferred drug-like properties. We are aware of this and will optimize efficiently and effectively, using our reiterative medicinal chemistry and PK knowledge to put forth the best inhibitor(s) for further development. We do not expect technical difficulties in executing the proposed experiments. All virological and animal models are well established in our laboratories. The series will be evaluated for their potential to move from *in vitro* studies to *in vivo* studies through their potency, efficacy, and drug-like properties and decisions will be based on criteria described in **Fig 7**. Of course, it is possible that compounds or chemical series cannot be optimized for acceptable drug-like properties with the preferred biological activity. We will consistently make **Go/No-Go** decisions as we execute lead optimization and discuss any issues or concerns we may have with the Project/Core leaders (Executive Committee), the Administrative Core, and SAC.

Aim 2. Identify novel Nuc DAAs with pan-flavivirus activity.

Rationale. Nuc-based drugs form the cornerstone of the current antiviral therapy, as they target the most conserved active sites of viral polymerases, leading to a broad spectrum of antiviral activity that suppresses different genotypes and serotypes of a given viral pathogen. In addition, Nuc inhibitors have a higher genetic barrier for resistance because mutations at the Nuc-binding site often are lethal for the viral polymerase. Indeed, NITD-008, an adenosine analog, inhibited all tested flaviviruses (*e.g.*, DENV, WNV, ZIKV, YFV, and Powassan virus) in cell culture and in mice; resistant viruses could not be selected in cell culture. Unfortunately, NITD-008 development was terminated because of toxicity in dogs when continuously dosed for two weeks[62,77,78]. The recent success of HCV antiviral therapy also highlights the importance of Nuc DAA. Sofosbuvir, a uridine-based drug that inhibits all genotypes of HCV, represents the core of the current HCV combination therapy[79]. Therefore, a Nuc-based antiviral approach has the potential to deliver a pan-flavivirus inhibitor.

2.1. Nuc collection. We will take a systematic approach to identify Nuc inhibitors by performing a very focused testing of the Nuc compounds from EIDD and (b) (3) (A) (b) that have not been tested previously against flaviviruses. The (b) (3) (A) (b) collection consists of Nucs that were previously synthesized for HCV program. The EIDD collection has ~800 compounds that were rationally designed as competitive, alternative substrate inhibitors of viral RdRp. The collection features Nuc analogs that have modified cyclic and acyclic carbohydrates coupled to natural nucleobases, modified nucleobases, which can participate in canonical Watson-Crick or Hoogsteen base pairing, and noncanonical nucleobases. The EIDD collection contains pairs of nucleoside analogs and their corresponding nucleoside triphosphate analogs. Testing of several leads has identified a potent inhibitor EIDD-1931 (its prodrug EIDD-2801) directed against SARS-CoV and MERS-CoV (**Project 1**) as well as alphaviruses (**Project 2**). **Indeed, our preliminary results showed that EIDD-1931 also has anti-flavivirus activity, with EC_{50} of 0.5-2.5 μM against DENV, ZIKV, and WNV; treatment with 3.3 μM of EIDD-1931 reduced production of all three flaviviruses by >10 fold in cell culture.** This success has prompted us to further evaluate the EIDD Nuc compounds for their activity against flaviviruses.

2.2. Two HTS assays to identify Nuc inhibitors of flaviviruses and their developmental approaches. For any Nuc analog to inhibit viral replication, the compound must be converted to Nuc triphosphate by host kinases inside cells; then, the Nuc triphosphate must be incorporated into viral RNA by the viral polymerase, leading to chain termination of RNA synthesis and/or mutation accumulation. Thus, two parameters determine

the antiviral activity of a Nuc compound: (i) the intrinsic ability of Nuc triphosphate to be recognized by the viral polymerase to terminate RNA synthesis; and (ii) the efficiency of cellular kinases to convert the Nuc to its triphosphate form. Using DENV-2, the Assay Core (**Core B**) will interrogate these two parameters of individual Nucs. Subsequently, we will evaluate 'hits' for breadth of activity against other DENV serotypes and related globally relevant flaviviruses (*e.g.*, ZIKV, WNV, YFV, and JEV) that we have in our laboratories.

(i) Testing nucleoside triphosphate analogs using a DENV-2 RdRp assay. We will use a fluorescence-based DENV-2 RdRp assay to test nucleoside triphosphate analogs from the EIDD and (b) (3) (A), collections. This effort will identify Nuc analogs whose triphosphate derivatives have intrinsic antiviral activity against DENV RdRp. Briefly, the nucleoside triphosphate analogs will be tested at 5 μM in the RdRp assay. Each compound will be tested in triplicate in a 96-well format as we described[52]. Compounds with >50% inhibition of RdRp activity will be retested in a 10-point, 2-fold-dilution series from 20 μM to 36 nM in triplicate to estimate the IC_{50} values.

(ii) Testing nucleoside analogs using a DENV-2 infection assay. A DENV-2 luciferase infection assay will be used to test Nuc analogs without phosphate from the Nuc collections[53]. We have chosen Huh7 cells for the infection assay because this cell line had sufficient levels of kinases for triphosphate conversion in HCV drug discovery[80-82], and the liver is one of the primary sites of DENV replication in patients[83]. Inhibitory activity in the viral infection assay suggests that the Nuc is efficiently converted to its triphosphate form inside cells, and that the Nuc triphosphate can inhibit viral RNA synthesis. We have previously reported the screening protocol of the DENV-2 luciferase infection assay[53]. Briefly, each nucleoside analog will be added to Huh7 cells at a final concentration of 10 μM in triplicate when the cells are infected with DENV-2 luciferase virus an MOI of 0.05. The infected cells will be assayed for luciferase activity at 48 h post-infection. Compounds with >50% inhibition of luciferase activity will be retested with 10-point, 2-fold-series dilutions from 30 μM to 59 nM in triplicates to estimate the EC_{50} values. For active compounds, their cytotoxicity will be evaluated in section **2.4**.

2.3. Optimization of Nuc inhibitors. The testing results of Nuc analogs and their triphosphate derivatives in the viral infection and RdRp assays, respectively, will guide our developmental approach. Results will be classified based on four possible outcomes: **Type I compounds** are active in both RdRp and virus infection assays, suggesting that these Nuc inhibitors are efficiently converted to their triphosphate forms and inhibit viral RNA synthesis. These compounds will be pursued as nucleosides for further development. (b) (3) (A), (b) (6) team will synthesize focused libraries around the identified hits using a late-stage glycosylation process by coupling nucleobases to a common carbohydrate intermediate[84]. **Type II compounds** are active in the RdRp assay, but inactive in the viral infection assay. These Nucs will be pursued as a monophosphate prodrug to improve its triphosphate conversion inside cells. This is because the first phosphorylation event often is the rate-limiting step of triphosphate conversion[85]. For these Nuc inhibitors, (b) (3) (A), (b) (6) team will synthesize phosphonate or phosphoramidate prodrugs. **Type III compounds** are inactive in the RdRp assay, but active in the viral infection assay, suggesting that the observed antiviral activity is likely due to off-target cytotoxicity or through an inhibitory activity of the compound metabolites. **Type IV compounds** are inactive in both RdRp and viral infection assays. We will not pursue type III and IV compounds.

2.4. Characterization of Nuc inhibitors. For each identified Nuc (including the known inhibitor EIDD-1931), we will characterize its antiviral parameters using DENV. Some experiments will be extended to other flaviviruses as indicated below.

(i) Antiviral potency in different cell types, including A549 and Vero. We have chosen these cells because they have active host kinases required for Nuc triphosphate formation[86]. We also will use PBMCs from rhesus macaques (RM), allowing us to assess the potential for success in RM model described in Aim 3. EC_{50} values derived from various cell types may differ because of the different efficiencies in triphosphate conversion.

(ii) Cytotoxicity. We will measure the CC_{50} values of each compound to estimate its cytotoxicity using CellTiter Glo that quantifies the intracellular ATP level (Promega). Cells will be treated with compounds (in triplicate with serial dilutions) for the same length of incubation time used in the viral infection assay. Only compounds with therapeutic indices (CC_{50}/EC_{50}) of >100 will be pursued in *in vivo* efficacy studies.

(iii) Antiviral spectrum. Apart from DENV-2, we will determine the EC_{50} values against the remaining three serotypes of DENV, as well as ZIKV, WNV, YFV, JEV, and TBEV. Antiviral assays are routinely performed on these viruses in our laboratories[50,62,87,88]. Since Nuc DAAs target the conserved active site of RdRp, we expect that some compounds will have pan-flavivirus activity. We also will test our top 'hits' against coronaviruses (**Project 1**), alphaviruses (**Project 2**), and influenza virus (**Project 4**), given the conservation of structure of viral RdRp. Compounds with broad-spectrum and potent antiviral activities will be prioritized.

(iv) Mechanism of inhibition. Most Nuc inhibitors exert their antiviral mechanisms through RNA chain termination[2]. We will perform a primer-extension experiment to examine if the Nuc terminates RNA synthesis once incorporated into the 3' end of an elongating RNA chain. The primer-extension assay will be performed

as we reported previously with some modifications[62]. Briefly, a ^{33}P-labeled 12-nucleotide primer will be annealed to an RNA template to initiate RNA synthesis in the presence of Nuc triphosphate, NTPs, and viral RdRp. The reaction products will be analyzed on an 8 M Urea denaturing PAGE. Autoradiographs of PAGE will reveal pre-terminated RNA products due to the incorporation of Nuc analogs.

(v) Resistance. We will attempt to select for drug resistant DENV and ZIKV in cell culture as described for the NS4B inhibitor in Preliminary Data. For each selected resistant virus, we will identify the mutation(s) by full-genome sequencing. Using our infectious clones of DENV and ZIKV[64,89], we will confirm the functions of the selected mutations by analyzing recombinant viruses containing the selected mutation(s). For each resistant virus, we will also evaluate the replication fitness *in vitro* (by comparing the replication kinetics of WT and resistant viruses on Huh7 and Vero cells), pathogenic potential *in vivo* (using the DENV AG129 and ZIKV hSTAT2-KI mouse models), and *Aedes aegypti* mosquito infectivity as described in Aim 1. Because Nuc inhibitors interact with viral RdRp, we expect resistance mutation(s) will map to the viral NS5 gene.

(vi) Mitochondrial RNA polymerase activity. One challenge of Nuc antiviral development is the unpredictable *in vivo* toxicity. Lessons learned from HCV Nuc development suggest that mitochondria RNA polymerase often accommodates Nuc analogs, leading to mitochondrial dysfunction[90]. The efficiency of Nuc incorporation into RNA product by mitochondria RNA polymerase has become a predictor for toxicity. Less than 3% incorporation efficiency of Nuc triphosphate (in comparison with the efficiency of its corresponding nature NTP) often is used as a cut-off for clinical development. Therefore, we will perform a mitochondria RNA polymerase assay to calculate the incorporation efficiency of our Nuc inhibitors as previously reported[90]. The mitochondria RNA polymerase will be purchased (Cayman Chemical). Compounds with ≥3% incorporation efficiency will be flagged as a safety liability for potential *in vivo* toxicity. In addition, we will extend the incubation time of the cytotoxicity assay to 7 days to further gauge the toxicity of the compounds.

(vii) *In vivo* efficacy. We will test the *in vivo* efficacy of promising Nucs in DENV-2, ZIKV, and WNV murine models. The treatments for DENV-2-infected AG129 mice or ZIKV-infected hSTAT2-KI mice will be performed as described in Aim 1. For WNV, we will infect 5-week-old C57BL/6 mice with 10^2 PFU of WNV-NY99 via the SC route; the infected mice will receive treatment starting at day 0, 1, 2, or 3 day after infection; the efficacy will be indicated by viral loads (in blood and organs), clinical disease, and histopathology[91]. The (b) (3) (A), (b) (6) laboratory developed the WNV pathogenesis model in C57BL/6 mice and has used it to test potential antiviral agents[92-94].

Importantly, ester prodrugs are commonly used to improve Nuc absorption and cellular membrane penetration. In humans, intracellular carboxylesterase degrades the ester moiety and releases the Nuc molecule for triphosphorylation. Because mice express a high level of secreted carboxylesterase 1c (*Ces1c*), which is absent in human plasma[95], the secreted carboxylesterase 1c in mouse plasma rapidly degrades the ester moiety of the prodrug before the compound enters cells. Therefore, for compounds with an ester prodrug moiety, we will use *Ces1c*$^{-/-}$ mice for efficacy testing. The *Ces1c*$^{-/-}$ mice are available from **Project 1** of the CETR, which has successfully used them to test the *in vivo* efficacy of GS-5734 (an ester phosphoramidate adenosine prodrug) against coronaviruses[96]. We will use the *Ces1c*$^{-/-}$ mice to test the efficacy of ester prodrugs against WNV and ZIKV. For testing ZIKV, the *Ces1c*$^{-/-}$ mice will be pre-treated with blocking antibodies against Ifnar1 at 24 h prior to viral infection; the pre-treatment with Ifnar1 antibodies facilitates ZIKV replication[97]; the compound treatment and efficacy analysis will be performed as described in Aim 1 (alternatively, we can cross the *Ces1c*$^{-/-}$ and hSTAT2-KI mice). Since DENV mouse model uses AG129 mice, and *Ces1c*$^{-/-}$ AG129 mouse is not available (and would require more extensive crossing), we will not test ester Nucs against DENV in mice. However, we will test non-ester Nuc compounds in AG129 mice, as we have previously done with NITD-008[62].

Expected outcomes and alternative approaches. In this Aim, we expect to identify pan-flavivirus Nuc inhibitors through focused systematic testing of novel compounds. The monophosphate prodrug approach will maximize the possibility of success to deliver a pan-flavivirus Nuc inhibitor for late preclinical development. The *in vitro* and *in vivo* experiments will reveal the safety and efficacy, antiviral mechanism, and resistance of the preclinical candidates. The proposed approach is established and has proven successful in antiviral discovery. The Project team has all the expertise to fully execute the plan and deliver the proposed milestones. **Dr. Pei-Yong Shi has >7 years of experience in Nuc antiviral development at Novartis where his team identified several Nuc and Nuc prodrug inhibitors of flaviviruses**[62,86,98-102]. (b) (3) (A), (b) (6) has developed seven clinically approved antiviral drugs, among which emtricitibine (a cytidine analog for HIV treatment) and brincidofovir (a prodrug of cidofovir for treatment of cytomegalovirus, adenovirus, smallpox, and ebolavirus) are Nuc inhibitors[103]. Furthermore, with the synergy on Nuc efforts from Projects 1, 2, and 4 as well as the collaboration with Gilead, we expect to rapidly achieve the milestones of Aim 2.

Aim 3. Evaluate combination therapy directed against flavivirus replication.

Rationale. Combination therapy is a common practice for anti-infective treatment to prevent or minimize drug resistance. Synergistic effects of combination therapy allow reduced dosing of individual drugs and therefore increased safety margins and decreased side effects of individual treatments. To apply this concept to flavivirus therapy, we will test therapeutic regimens combining DAAs with distinct viral targets from Aims 1 and 2 as well as other known inhibitors. For each pair of inhibitors, we will address three parameters for combination therapy: (i) synergistic or additive effects; (ii) resistance emergence; and (ii) *in vivo* validation.

3.1. Synergistic or additive effect. We will test two combinations regimens: (i) DENV C inhibitor + Nuc, (ii) ZIKV NS4B + Nuc. To examine whether combination of two compounds has synergistic or additive effects, we will infect Huh7 cells with luciferase DENV-2[53] or luciferase ZIKV[64] at an MOI of 0.5. Each compound pair will be added immediately to the infected cells in a checkerboard set of concentrations. We will test each compound at seven two-fold-diluted concentrations (excluding a control without compound) centered on the EC_{50} value. At 24-48 h post-infection, the infected cells will be measured for luciferase activity. Each experiment will be performed in quadruplet to ensure reproducibility. The levels of luciferase inhibition will be used to determine if the drug combination is synergistic or additive. Specifically, we will use Chalice Analyzer to calculate the Loewe excess, Bliss excess, and HSA excess values[104-106]. These parameters are used commonly to indicate the excess percentage inhibition; the excess percentage inhibition is calculated by deducting the expected percentage inhibition values of various combinations, assuming non-synergy pairing in various models, from the experimental percentage inhibition values. These data will allow us to calculate isobolograms, synergy scores, and best combination indices (CI) for each pair of inhibitors. A synergy score of >1 and CI of <1 indicate that a combination treatment has a synergistic effect, whereas a synergy score of 1 and CI of 1 indicate that a combination treatment has an additive effect (34). To assess whether synergy could be achieved at high inhibition levels, we will set the isobolograph level at 0.9 to capture meaningful synergy with 90% viral reduction (equivalent to one $\log_{10}$ reduction of viral replication). We also will perform cytotoxicity tests at the same combination concentrations to exclude the possibility that the observed synergistic or additive effects are due to cytotoxicity. Using this approach, we previously showed a synergistic antiviral effect of two inhibitors (a guanosine analog and an inhibitor of guanosine synthesis) when treating DENV infection[99].

3.2. Resistance emergence. To examine resistance development during combination therapy, we will infect Huh7 cells with WT DENV-2 or ZIKV at an MOI of 0.1. The infected cells will be treated with a pair of DAAs, each at 1× their EC_{75} concentrations. Concurrently, the infected cells also will be treated with the individual compounds alone at 2× their EC_{75}. Mathematically, such equipotent combinations should have the same level of antiviral activity if each compound is considered to have the same biological characteristics. This method has been used successfully to estimate resistance for HCV combination therapy[80]. At 48-72 h post infection, the culture supernatants will be used to infect naïve Huh7 cells in the presence of the same treatment regimen. We will continue passaging with treatment for 10 rounds. If CPE develops prior to round 10, we will confirm the resistant phenotype of the selected viruses by comparing their sensitivities to compounds with the WT virus. Once the resistance phenotype has been confirmed, we will sequence the genomes of the selected viruses. Based on our previous experience with Nuc inhibitors[62,100,101], we expect that treatment with combination of "DENV C inhibitor + Nuc" or "ZIKV NS4B + Nuc" will not generate resistant virus. This is because Nuc inhibitor has a high barrier of resistant emergence. Treatment with inhibitors of DENV C or DENV NS4B alone is expected to generate resistant viruses[46,50,51].

3.3. *In vivo validation.* We will expand the *in vitro* combination efficacy to *in vivo*. Compounds from the above cell culture experiments and with favorable PK/PD profile will be tested first in mice. Based on the PK profiles, two doses will be tested for each compound in the combination regimens: a saturating dose (the dose that yields the highest compound exposure in serum) to test the maximal potential of inhibition, and a non-saturating dose to observe the synergistic/additive effect with another compound. The antiviral efficacy will be indicated by the parameters of infection and pathogenesis described in Aim 1. Finally, based on the mouse efficacy results, the most promising DAA combination (with high doses of individual compounds) will be validated in a non-human primate (NHP) model of ZIKV infection.

ZIKV infection in the genus *Macaca* has been extensively characterized by viremia, viruria, and the persistence of viral RNA within multiple tissue types for more than 1 month after infection[107-112]. The model also supports virus transmission to the fetus during pregnancy as well as aspects of fetal pathogenesis[113,114]. We will test the NHP efficacy of the candidate ZIKV inhibitor combination that shows greatest activity in mice. In the event that combinations do not display greater efficacy in mouse studies compared to individual compounds, monotherapy experiments will be assessed with the single best ZIKV inhibitor. We have chosen the ZIKV NHP model for testing antiviral activity because, compared with DENV and WNV NHP models, the ZIKV model recapitulates many more virologic and pathologic aspects of human disease. We will first evaluate the PK of individual compounds in rhesus macaques (RM). A proportionally scaled efficacious dose will be administrated via optimal route (based on previous mouse results) daily to adult (4+ years) RMs for 3 days. Blood will be

collected from animals at 24 h intervals; compound levels of initial exposure (at the first 24 h) and accumulation (over the 3-day period) will be detected by HPLC. Blood chemistry panels and complete blood counts will be performed to evaluate effects of drug treatment. Based on the PK results, we will dose the compounds to achieve serum exposure levels of $3\times EC_{90}$ for efficacy test. Three cohorts (n=6 each) will be subjected to ZIKV challenge (10^5 PFU epidemic ZIKV PRVABC59 strain via SC). The first cohort will begin drug treatment at day 0 (coincident with infection), the second cohort will begin treatment at day 2 post-infection, while the third will serve as untreated controls. Dose, frequency, and route will be informed by prior PK tests and proceed through 7 days post-infection. After infection, RMs will be evaluated daily for changes in body temperature and clinical signs, including fever, lymphadenopathy, and rash. We will collect peripheral blood and urine after challenge at days 1–6, 10, and 14 post-infection. Blood will be processed into serum, plasma, and PBMC fractions for virus titration. Animals will be euthanized at day 14 post-infection. At necropsy, we will collect tissues (joints, muscle, lymph nodes, blood, organs, cerebral spinal fluid, brain, spinal cord, and tissues of the urogenital and digestive tracts) and determine viral loads to assess disease severity. Each tissue will be divided and: (i) fixed and embedded for IHC; (ii) solubilized in Trizol for RNA extraction; and (iii) homogenized for live virus titration by plaque assay. Total RNA will be purified from serum, urine, and saliva. Viral genomes will be quantified by qRT-PCR as previously described[114]. In parallel, infectious virus will be measured by focus-forming assay on Vero cells or by culture on C6/36 cells and immunofluorescent detection. The isolated viruses from the different tissues will be stored for further analysis including sequencing to identify escape mutations that may be associated with the drug treatment. Based on our previous studies, this experimental design will give us 87% power to detect a 1.5 log change in mean viral load in serum or tissue (α=0.05). These experiments will be carried out at the Oregon National Primate Research Center, of which (b) (3) (A), (b) is an affiliated scientist. Our group has extensive experience with the RM model of ZIKV infection[110,114], and we do not anticipate problems with executing or interpreting these experiments.

Expected outcomes and alternative approaches. The proposed experiments will determine the synergistic/additive effect and the resistance potential of combination therapy. The results will allow us to compare the differences in these parameters among distinct combinations (*i.e.*, C inhibitor + Nuc and NS4B inhibitor + Nuc). Beside the proposed combinations, we may evaluate new broad-spectrum inhibitors identified by other Projects in AD3C, and potentially include them in combination regimens, if appropriate. **These experiments, including NHP studies, will generate critical information to guide future clinical development of flavivirus therapy.** We have the required experimental systems and experience to execute the proposed experiments.

Timeline and milestones

Aim	Tasks and milestones	Year 1				Year 2				Year 3				Year 4				Year 5			
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
1	Establish SAR for DENV C inhibitor																				
	Determine co-crystal structures of C and inhibitor																				
	Determine EC_{50} and CC_{50} of C and NS4B inhibitors																				
	Resistance analysis and antiviral spectrum																				
	Analyze PK/PD of C and NS4B inhibitors																				
2	Perform HTS of Nuc libraries																				
	MedChem prodrug																				
	Determine EC_{50}, CC_{50}, and toxicity																				
	Resistance analysis and antiviral spectrum																				
	Determine mouse efficacy																				
3	Perform combination efficacy in cells																				
	Perform resistance analysis																				
	Determine EC_{50} and CC_{50}																				
	Determine mouse efficacy																				
	Determine NHP efficacy																				

PHS Human Subjects and Clinical Trials Information

OMB Number: 0925-0001 and 0925-0002

Expiration Date: 03/31/2020

Are Human Subjects Involved	○ Yes ● No
Is the Project Exempt from Federal regulations?	○ Yes ○ No
Exemption Number	❑ 1 ❑ 2 ❑ 3 ❑ 4 ❑ 5 ❑ 6 ❑ 7 ❑ 8
Does the proposed research involve human specimens and/or data	○ Yes ● No
Other Requested information	

VERTEBRATE ANIMALS

(b) (3) (A), (b) (6), M.D., Ph.D.
Washington University School of Medicine

Washington University animal use approval number: (b) (3) (A), (b) (6)

1 and 2 (Description and Justification). Despite the significant morbidity associated with flavivirus infection, there are no approved therapeutics available for use with any virus member of the genus. In this application, we will use animal models (WT C57BL/6 mice and human STAT2-KI mice) to test the efficacy of newly selected anti-flavivirus small molecule (nucleoside and capsid inhibitors) against Zika virus. Significant effort will be initiated by the (b) (3) (A), (b) (6) laboratory in the Flavivirus Project to defining the level of protection of individual small molecule candidates and ultimately combinations that show synergy and prevent emergence of drug resistance. After challenge, we will perform detailed analysis of morbidity, viral burden, and as needed immunohistochemical analyses on treated and infected animals. The primary goal of this project is to define which optimized small molecules show elite protective activity against a broad range of flaviviruses, using Zika virus as a model. In addition, we will evaluate the protective effects of small molecules on congenital Zika virus syndrome, which we have modeled recently in both WT mice treated with an anti-Ifnar1 antibody and in human STAT2-KI mice. **We note however, that implementation of small molecule antiviral drugs for use in pregnancy will require extensive testing** (beyond the scope of this project) to ensure no possibility of drug-induced teratogenicity. Nonetheless, we think this goal is important and worth testing in pre-clinical models in the same way that nucleoside inhibitors are used to prevent HIV acquisition during pregnancy.

We have chosen to evaluate drug candidates against Zika virus because of the well-characterized pathogenesis model that resembles many features of human disease. Significant effort will be initiated by the (b) (3) (A), (b) (6) laboratory to defining which of the lead small molecules developed by this Project shows the best activity in vivo activity. After challenge, we will perform detailed analysis of morbidity, viral burden, and as needed immunohistochemical analyses on vehicle and small molecule treated, infected animals. The primary goal of this project is to define which small molecules show elite protective activity (and little emergence of resistance) against a broad range of flaviviruses, using Zika virus as one of the in vivo models, along with extensive in vitro studies against other flaviviruses. This information will be provided to the CETR group, as we collectively evaluate small molecules against multiple emerging RNA viruses of global concern. We will evaluate the protective effects of lead candidate molecules in male and female mice including for impacts on congenital Zika virus syndrome, which we have modeled recently in both WT mice treated with an anti-Ifnar1 antibody and in human STAT2-KI mice.

To understand the expression of a disease and level of protection conferred by a given antiviral therapy, the interaction between the virus and the host must be studied in vivo. Cell tropism, histopathology, immune system activation, and the evolution of infection and resistance over time are best assessed in an animal model. Analysis of the efficacy of an antiviral in a physiological context requires direct experimentation in animals. Although significant information can be gained from tissue culture *in vitro* experiments (*e.g.*, EC_{50}, CC_{50}, Selectivity, in vitro resistance, breadth of activity, solubility, druggability, SAR), the definitive inhibitory activity must be confirmed in animal models. The disease models in WT and human STAT2-KI mice for Zika virus will serve as primary models of infection, for which we can evaluate drug candidates. There is no substitute for animal models as a way to identify antivirals against infection and disease. Thus, while transformed cell lines have value for some things, they cannot be used to directly study a disease process, which occurs only *in vivo*. It would be impossible to undertake the proposed experiments without using mice, given their throughput and development of disease after Zika virus (and other flavivirus) infection. Mice are used because the available strains provide key information on the efficacy of antivirals against flaviviruses, and these studies can be performed in scale to allow rigorous statistical analysis.

Infection studies in the (b) (3) (A), (b) (6) laboratory will be performed with WT and human STAT2-KI transgenic C57BL/6 mouse strains; the (b) (3) (A), (b) (6) laboratory has developed these as key models of infection with different Zika virus disease outcomes depending on sex, age, and route of challenge. C57BL/6 mice ($H\text{-}2^b$) are used because of the large number of immunological tools available for profiling responses. In some experiments, WT and human STAT2-KI mice (male and female, beginning at 6 weeks of age) will be treated with antiviral compounds prior to challenge; in other experiments, antiviral agents will be administered at different days after infection. In additional studies, female WT and human STAT2-KI mice will be mated with WT male mice. Pregnant mice will then be challenged at E6.5, given lead drugs, and we will analyze protection at E13.5 or at birth. This model is directly relevant to protection against Zika congenital syndrome.

The animal studies will vary in number in each year as we perform intensive down-selection and apply Go/No-Go decisions. For example, in the first two years of this proposal, we expect to use ~700 mice per year (50 breeders, 200 male-female combinations for pregnancy challenge, and 500 mice for testing the target set of hits from the capsid and polymerase inhibitors. In FY3-5, the number of animals required will fall (250 to 500 total mice per year) after Go/No-Go decisions are applied, as we move to our top hits and the combinations of these. This number of mice needed to complete these studies was derived from our vast experience in designing viral pathogenesis and drug protection experiments with mice. The numbers reflect a series of factors including: (a) the number needed to attain statistical power for an outcome under study; (b) the need to replicate in vivo experiments in independent replicates, and (c) the scale of the difference (e.g., more animals will be required to attain significance if the difference in outcome (e.g., viral titer) is smaller); (d) the number needed to attain statistical power for an outcome under study - power calculations are provided below. Despite our requirement for the use of a large number of mice, we are aware of the need to restrict experiments to those that directly address scientific questions that cannot be answered *in vitro* or in cells. We plan to as many of the experiments in cell culture as is scientifically feasible to prioritize 'hit' down-selection prior to initiating in vivo studies.

3. Veterinary care. Veterinary care is provided by the Division of Comparative Medicine (DCM) at Washington University School of Medicine by their dedicated and trained staff. DCM works closely with the Animal Studies Committee and investigators to design and implement protocols assuring humane treatment and reasonable use of animals. All experiments are designed with attention to the humane treatment and reasonable use of animals. In addition, all veterinary care staff are trained in handling of animals infected with BSL-2 and BSL-3 agents. This is described in more detail in the Biosafety, Biohazard, and Biocontainment sections.

4 and 5 (Provisions to minimize discomfort, distress, pain, and injury and plan for euthanasia). All mice will be housed in our animal facility at Washington University. To minimize discomfort and distress, all mice will be anaesthetized and sedated with ketamine and xylazine prior to inoculation with Zika virus according to strictly defined protocols. Sedation will not be used for immunization alone, as per protocol. Inoculated and immunized mice will be monitored for signs of distress or discomfort. Mice will be observed on a daily basis for signs of weight loss, lethargy, or inability to eat or drink. Distressed or moribund mice will be euthanized using CO_2 asphyxiation after ketamine and xylazine anaesthesia to decrease suffering. We will use criteria for distress as outlined in the guidelines provided by the Washington University Animal Studies Committee and the American Veterinary Medical Association. Mice that are to be bled will be anaesthetized. Blood will be collected by a Washington University Animal Studies Committee approved technique: lateral saphenous and dorsal tail phlebotomy. These techniques will be performed at a frequency of no greater than every other day with a volume not to exceed 50 µl.

Experimental design of studies with mice. We have consulted a biostatistician in our Department of Medicine to structure and design the animal studies. The (b) (3) (A), laboratory has published phenotypic characterization of more than 40 different KO mouse strains in the context of viral pathogenesis and drug treatment, and thus has extensive experience in the design and analysis of animal studies. Moreover, they have worked with (and published studies) with Zika virus including during pregnancy. We have powered the studies to detect differences in absolute survival of 50% or greater, differences in the time to mean death of 2 days, and viral burden differences of approximately 5-fold in a given tissue. For challenge studies and virological work-up, we will harvest tissues from WT and human STAT2-KI transgenic mice that are treated with drugs and then infected with the same stock of Zika virus (that has been frozen and aliquoted). A typical experiment (would be as follows: Age (*e.g.*, 6 weeks of age) and sex-matched (we use both males and female mice) WT or human STAT2-KI mice in groups of 4 to 5 are treated with drug or vehicle at a given dose and/or frequency (defined by PK studies). Mice (tagged and genotyped) are co-housed to minimize differential effects of the microbiome. Serial bleeds are performed. Subsequently, animals are inoculated with Zika virus (Dakar 41525). Animals are monitored daily until the endpoint (*e.g.*, euthanasia and harvesting of tissues) is reached. This exact experimental design will be repeated at least three independent times on different days to attain an n of 12 to 15 per cohort. In some cases, if we anticipate smaller phenotypic differences, additional repetition of experiments may be performed with larger groups of mice. As part of this analysis, we also compare outcomes with male and female mice to determine whether sex-based differences in a phenotype exist. If this is observed, we stratify the analysis and increase the experimental numbers to confirm such findings.

For pregnancy studies, female mice will be mated with WT male mice. At E6.5, dams will be inoculated with Zika virus and treated with drug or vehicle. At E13.5 or E19.5 (birth), animals will be euthanized, and virological (maternal blood, spleen, and brain; fetal placenta, body, and head) analyses will be performed.

Power calculations used for complete proposal: As detailed above, experiments in this proposal use a mouse model to define protection against flavivirus infection conferred by inhibitors. To determine mouse group sizes for virological or immunological studies, power analysis was performed using commonly accepted values for type I error (0.05) and power (80%). The values for population variance (25-fold for virological studies; and 50% for survival studies) were based on existing data sets from our long-term experience with virus infection studies in C57BL/6 mice.

Pei-Yong Shi, Ph.D.
University of Texas Medical Branch (UTMB)

UTMB approval number: 1708051

1. Description of animal use. Dengue virus (DENV) is the most important mosquito-borne viral pathogen with over 390 million infections per year. No clinically approved antiviral drug is currently available to treat DENV and other flavivirus infections. Therefore, the development of flavivirus antiviral therapy is a public health priority. The goal of this proposal is to develop direct antiviral agents (DAAs) for flavivirus therapy. In this application, we will use AG129 mouse to test the efficacy of newly selected anti-flavivirus small molecule (*e.g.*, capsid, NS4B, and nucleoside inhibitors) against DENV. The (b)(3)(A) (b)(6) laboratory will use the well-established DENV AG129 mouse model to define the level of protection of individual inhibitors and ultimately combinations that show synergy as well as to prevent emergence of drug resistance. After DENV infection, we will perform detailed analysis of morbidity, viral burden, and immunohistochemical analyses on treated and infected animals. The primary goal of this project is to identify compounds with potent efficacy against DENV and possibly other flaviviruses. Combination regimens (*e.g.*, nucleoside and capsid inhibitors) will also be tested for synergistic or additive effects, and for mitigation of drug resistance in the DENV AG129 mice. The *in vivo* mouse results of efficacy and resistance will be compared with the *in vitro* cell culture results. This information will be provided to the CETR group, as we collectively evaluate small molecules against multiple emerging RNA viruses of global concern.

Experimental design of studies with mice. Advanced compounds with favorable PK profiles will be examined for their *in vivo* efficacy in the AG129 (lacking IFN-α/β and IFN-γ receptors) DENV mouse model. AG129 mice will be housed at UTMB in an A-BSL2 animal facility. Groups of 10 to 15 8-week-old mice will be inoculated for each condition and equal numbers of negative and positive controls will be included. For experiments designed to appreciate smaller effects on efficacy parameters (see below), greater numbers of mice will be used to increase the power of the study. Each mouse will be inoculated with 4×10^6 PFU of DENV-2 strain TSV01 via the IP route. Initially, we will test the compound efficacy by starting the treatment within 15 min of viral infection. If efficacy is achieved, we will pursue post-exposure efficacy by delaying the start of treatment at 12, 24, 36, and 48 h post-infection. We will measure four parameters to indicate compound efficacy: (i) Viremia at days 3 and 4 post-infection using plaque assay on BHK-21 cells; (ii) inflammatory cytokine and chemokine levels of TNF-α, IL-6, IFN-γ, MCP-1, and IL-12p70; (iii) body weight loss; and (iv) survival. The dosing regimens of compounds will be determined by their PK profiles. This is a standard protocol that has been widely used for evaluating antiviral activity *in vivo*. Animals are monitored daily until the endpoint (*e.g.*, euthanasia) is reached. This exact experimental design will be repeated at least three independent times on different days.

2. Justification of animal use. The antiviral effects on viremia, cell tropism, histopathology, immune system activation, and the evolution of infection and resistance over time are best assessed in an animal model. Analysis of the efficacy of an antiviral in a physiological context requires direct experimentation in animals. Although significant information can be gained from tissue culture *in vitro* experiments (*e.g.*, EC_{50}, CC_{50}, Selectivity, *in vitro* resistance, antiviral soectrum, solubility, druggability, and SAR), the definitive inhibitory activity must be confirmed in animal models. The DENV-2 AG129 mouse model will be used as a primary model to evaluate drug candidates for DENV. No substitute for animal models is available as a way to identify antivirals against infection and disease.

The member of A129 mouse for each year will decrease as we down-select and apply Go/No-Go decisions. In the first three years of this project, we expect to use ~400 mice per year for testing the hits from the capsid, NS4B, and RdRp inhibitors. In year 4, the number of animals required will fall to ~200 as we down select to top hits and the combinations of these. No AG129 mouse experiments will be performed in year 5 when the most advanced compounds are tested in non-human primates. The proposed number of mice is estimated on the basis of our previous experience in antiviral and vaccine development. The animal numbers are determined by (i) the number needed to attain statistical power for an outcome, (ii) the need to replicate *in vivo* experiments in

independent replicates, and (iii) the scale of the difference (*e.g.*, more animals are required to attain significance if the difference in outcome is smaller). Despite the requirement for a large number of mice, we will restrict experiments to those that directly address scientific questions that cannot be answered *in vitro* or in cells. In addition, we will maximize cell culture experiments to prioritize hits. This experimental flow will allow us minimize the number of mouse experiments.

3. Veterinary care. At UTMB, all animals are housed in a veterinarian supervised, approved animal facilities that have been awarded Accreditation by the American Association for Accreditation of Laboratory Animal Care Council. All research are conducted in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals, and adhere to principles stated in the "Guide for the Care and Use of Laboratory Animals" (National Research Council, 1996). The UTMB Animal Care Center is under the direction of a Doctor of Veterinary Medicine and staffed by veterinarians with training and experience in laboratory animal medicine and surgery, clinical care and diagnostic pathology." Specifically, infected mice will be housed in microisolator cages within the ABSL-2 at UTMB and monitored at least daily and observed for signs of disease caused by inhibitors or viral infections. Animals will be monitored at least once per day and twice per day if illness is expected. Blood samples will be taken via the saphenous vein, retro-orbital sinus, or by cardiac puncture (for terminal bleeds, under isoflurane anesthesia) to determine viremia, cytokines and chemokines.

4. Procedures for ensuring that discomfort, distress, pain, and injury. Analgesics cannot be used on mice because they might have unintended physiological effects that would influence pathogenesis, as measured in our histopathology analyses, and the ability to detect protective effects of compound treatment. However, animals will be euthanized if they exceed a score of 11 on the following quantitative assessment of pain and distress chart:

Quantitative Assessment of Pain

Independent Variable Score

Body Weight:
0 - - Normal
1 - - <10% weight loss
2 - - 10-15% weight loss, eating
3 - - >20% weight loss, not eating
Appearance:
0 - - Normal
1 - - Lack of grooming
2 - - Coat rough, possible nasal and or ocular discharge
3 - - Coat very rough, abnormal posture, pupils enlarged
Clinical Signs:
0 - - Normal
1 - - Small change of potential significance
2 - - Temperature rise 1-2, 30% rise in respiratory/heart rates
3 - - Temperature change >2, 25% rise in respiratory/heart rates (or markedly reduced/shallow)
Unprovoked Behavior:
0 - - Normal
1 - - Minor changes
2 - - Abnormal behavior, less mobile, less alert, inactive when activity expected
3 - - Unsolicited vocalization, extreme self-mutilation
Behavior Response to External Stimuli (palpation or injection sites):
0 - - Normal
1 - - Minor exaggerated response
2 - - Moderate abnormal response
3 - - Violent response
Total score: _____

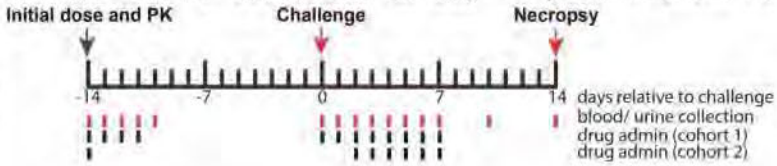
5. Euthanasia. Mice will be euthanized with an intravenous overdose of pentobarbital, or by exposure to CO_2 until breathing and heartbeat are absent. Death will be confirmed by open chest examination. These are effective, safe methods consistent with the Panel on Euthanasia of the American Veterinary Medical Association.

VERTEBRATE ANIMALS: OHSU / Vaccine and Gene Therapy Institute

Non-human primates: A nonhuman primate model will be used to study Zika virus pathogenesis, immunity and viral distribution following treatment with anti-viral compounds developed in this proposal. The use of rhesus macaques (RM) is critical to the experiments described in this application. All animal studies require prior approval by the Oregon Health and Science University (OHSU) animal review committee, and the proposed experiments are currently under review by the IACUC committee. The performance site for immunization studies using rhesus macaques will be OHSU and the Oregon National Primate Research Center (ONPRC) (PHS/OLAW Animal Welfare Assurance # A3304-01).

(1) Description of Procedures: A total of 18 rhesus macaques (RM, *Macaca mulatta*), approximately equal numbers male and female, will be used to conduct vaccine efficacy studies of the most effective drug or drug combination (one nucleoside analog and one non-nuc inhibitor) during year 5 of the proposed funding period. Animals will be sedated with ketamine hydrochloride (5-10 mg/kg body weight) before any procedure under the direction of a veterinarian in dedicated aseptic surgery facilities. Toxicity, PK profile, and efficacy will be determined in mice prior to RM experiments. These experiments will also direct formulation, dose, and route of administration. In order to verify bioavailability of the compounds in the RM, we will administer drug to 6 animals for 4 days, and collect blood from treated animals at 24 h intervals. Compounds will be detected in plasma by HPLC. We will dose the compounds to achieve serum exposure levels of $>EC_{90}$. Doses will be modified if initial drug exposure proves too low.

Three cohorts (n=6 each) will be subjected to ZIKV challenge (10^5 PFU ZIKV PRVABC59 via SC). The first cohort will begin drug treatment at day 0 (coincident with infection), the second cohort will begin treatment at d 2 post-infection, while the third will serve as untreated controls. In cohorts 1 and 2, animals will be further treated once daily after initial dose through 7 dpi. Following challenge, RMs will be evaluated daily for changes in body temperature and clinical signs, including fever, lymphadenopathy, and rash. We will collect peripheral blood and urine after challenge at days 1–6, 10, and 14 post-infection. Blood will be processed into serum, plasma, and PBMC fractions for virus titration. Animals will be euthanized at day 14 post-infection. At necropsy, we will collect tissues (joints, muscle, lymph nodes, blood, organs, cerebral spinal fluid, brain, spinal cord, and tissues of the urogenital and digestive tracts) and determine viral loads to assess disease severity.



(2) Justification for the Use of Animals and Species: There is no alternative to the use of animals in these studies. Mathematical modeling cannot provide the desired information on Zika virus pathogenesis and immune responses outlined in this proposal – only *in vivo* experimental studies can do this. Rhesus macaques were selected for inoculation/pathogenesis studies because they are susceptible to infection with Zika virus and disease; the first strain of Zika virus was isolated from a febrile RM. RM make a good model of human:Zika virus pathogenesis. This is especially true when modeling immunity and Zika disease. Our Zika:NHP model is critical for preclinical testing of therapeutics and vaccines directed against Zika virus. Further, sites of viral persistence and replication appear different in the macaque (and human) than in interferon-deficient mice. Numbers of animals were determined by power analysis. Given our preliminary data on viremia and immune response to ZIKV in the RM, n=6 per group will give us ~87% power to detect 1.5 log difference in peak viremia or tissue RNA load following challenge (α=0.05).

(3) Minimizing Discomfort, Distress, Pain and Injury: The laboratory animal care program at the ONPRC is fully accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC). The Division of Comparative Medicine is headed by a veterinarian who is certified as a Diplomat by the American College of Laboratory of Animal Medicine. This individual is also appointed as the institutional attending veterinarian for regulatory compliance. Veterinary care and research animal professional services are provided by the Head of the Division, certified as a Diplomat by the American College of Laboratory Animal Medicine, and an Associate Veterinarian. The Division provides service in support of the animal care program. All major surgical procedures on nonhuman primates are performed by or under the direction of a veterinarian in dedicated aseptic surgery facilities. Diagnostic pathology support is provided by a veterinarian who is certified as a Diplomat by the American College of Veterinary Pathologists. Laboratory animal care is provided by the Division, which is supervised by a veterinarian. Animal husbandry care and technical and administrative

support are provided by a complement of trained and competent individuals. The majority of the animal care staff is certified by the American Association for Laboratory Animal Science at the Assistant Laboratory Animal Technician, Laboratory Animal Technician, or Laboratory Animal Technologist level. Some of the technical personnel have college degrees or State Animal Health Technician certification. Also included is a diagnostic clinical pathology laboratory staffed by two certified Medical Technologists. All animals are observed at least twice a day by trained animal technicians. Ill animals are reported and treated according to accepted veterinary medical practices. Records documenting all medical procedures as well as experimental use, reproductive history, and demographic data are maintained in a computerized database for the life of the animal. All nonhuman primates housed in indoor or indoor/outdoor facilities are weighed monthly and TB tested semi-annually. The animal husbandry program includes daily sanitation of the waste collection pans. Cages are sanitized in a central cage washer at least once every 2 weeks. Nonhuman primates are provided fresh water and routinely fed commercially prepared primate feed milled within the past 90 days. This feed is supplemented daily with fruit and special diets prepared onsite. Ketamine HCl 5-10 mg/kg or Telazol® 3-8 mg/kg will be used to induce anesthesia for all routine non-invasive clinical procedures associated with the study protocols such as blood and therapy administration as well as clinical examinations or treatment. Blood sample volumes will be limited to 10% of total blood volume calculated at 54ml/kg body weight in any 21-day period of time.

Following Zika virus infection, animals will be evaluated for clinical signs of disease on a daily basis by the Division of Comparative Medicine staff. The clinical veterinarian will be responsible for determining if the animal is experiencing any pain or suffering from viral infection. At the discretion of the veterinarian and in consultation with the principal investigator, animals will receive treatment for treatable conditions, related or unrelated to the experimental protocol. If pain or suffering associated with viral infection or other causes cannot be alleviated through appropriate treatment, animals will be euthanized after consultation between the clinical veterinary and investigative staff.

(4) Euthanasia: Euthanasia will be by sodium pentobarbital overdose (greater than 50mg/kg body weight) followed by exsanguination. This method is consistent with the recommendations of the American Veterinary Medical Association 2013 Panel on Euthanasia.

REFERENCES

1 Pierson, T. C. & (b) (3) (A), M. S. *Flaviviruses p. 747-794. In D.M. Knipe and P.M. Howley (ed), Fields virology, 6th., vol. 1.*

2 De Clercq, E. & Li, G. Approved Antiviral Drugs over the Past 50 Years. *Clin Microbiol Rev* **29**, 695-747, doi:10.1128/CMR.00102-15 (2016).

3 Bhatt, S. *et al.* The global distribution and burden of dengue. *Nature* **496**, 504-507, doi:10.1038/nature12060 (2013).

4 Kyle, J. L. & Harris, E. Global spread and persistence of dengue. *Annual Review of Microbiology* **62**, 71-92, doi:10.1146/annurev.micro.62.081307.163005 (2008).

5 Lim, S. P. *et al.* Ten years of dengue drug discovery: Progress and prospects. *Antiviral Res* **100**, 500-519, doi:10.1016/j.antiviral.2013.09.013 (2013).

6 Sabchareon, A. *et al.* Protective efficacy of the recombinant, live-attenuated, CYD tetravalent dengue vaccine in Thai schoolchildren: a randomised, controlled phase 2b trial. *Lancet* **380**, 1559-1567, doi:10.1016/S0140-6736(12)61428-7 (2012).

7 Villar, L. *et al.* Efficacy of a tetravalent dengue vaccine in children in Latin America. *N Engl J Med* **372**, 113-123, doi:10.1056/NEJMoa1411037 (2015).

8 Capeding, M. R. *et al.* Clinical efficacy and safety of a novel tetravalent dengue vaccine in healthy children in Asia: a phase 3, randomised, observer-masked, placebo-controlled trial. *Lancet* **384**, 1358-1365, doi:10.1016/S0140-6736(14)61060-6 (2014).

9 Hadinegoro, S. R. *et al.* Efficacy and Long-Term Safety of a Dengue Vaccine in Regions of Endemic Disease. *N Engl J Med* **373**, 1195-1206, doi:10.1056/NEJMoa1506223 (2015).

10 Whitehead, S. S. Development of TV003/TV005, a single dose, highly immunogenic live attenuated dengue vaccine; what makes this vaccine different from the Sanofi-Pasteur CYD vaccine? *Expert review of vaccines* **15**, 509-517, doi:10.1586/14760584.2016.1115727 (2016).

11 Shan, C. *et al.* Zika Virus: Diagnosis, Therapeutics, and Vaccine *ACS Infectious Diseases* **2**, 170-172 (2016).

12 Aliota, M. T. *et al.* Zika in the Americas, year 2: What have we learned? What gaps remain? A report from the Global Virus Network. *Antiviral Res*, doi:10.1016/j.antiviral.2017.06.001 (2017).

13 Roundy, C. M. *et al.* Variation in Aedes aegypti Mosquito Competence for Zika Virus Transmission. *Emerg Infect Dis* **23**, 625-632, doi:10.3201/eid2304.161484 (2017).

14 Thangamani, S., Huang, J., Hart, C. E., Guzman, H. & Tesh, R. B. Vertical Transmission of Zika Virus in Aedes aegypti Mosquitoes. *Am J Trop Med Hyg* **95**, 1169-1173, doi:10.4269/ajtmh.16-0448 (2016).

15 Musso, D. *et al.* Potential sexual transmission of Zika virus. *Emerg Infect Dis* **21**, 359-361, doi:10.3201/eid2102.141363 (2015).

16 Kuehnert, M. J. *et al.* Screening of Blood Donations for Zika Virus Infection - Puerto Rico, April 3-June 11, 2016. *MMWR Morb Mortal Wkly Rep* **65**, 627-628, doi:10.15585/mmwr.mm6524e2 (2016).

17 Motta, I. J. *et al.* Evidence for Transmission of Zika Virus by Platelet Transfusion. *N Engl J Med* **375**, 1101-1103, doi:10.1056/NEJMc1607262 (2016).

18 Besnard, M., Lastere, S., Teissier, A., Cao-Lormeau, V. & Musso, D. Evidence of perinatal transmission of Zika virus, French Polynesia, December 2013 and February 2014. *Euro Surveill* **19** (2014).

19 Oliveira Melo, A. S. *et al.* Zika virus intrauterine infection causes fetal brain abnormality and microcephaly: tip of the iceberg? *Ultrasound Obstet Gynecol* **47**, 6-7, doi:10.1002/uog.15831 (2016).

20 Calvet, G. *et al.* Detection and sequencing of Zika virus from amniotic fluid of fetuses with microcephaly in Brazil: a case study. *The Lancet. Infectious diseases* **16**, 653-660, doi:10.1016/S1473-3099(16)00095-5 (2016).

21 Dick, G. W., Kitchen, S. F. & Haddow, A. J. Zika virus. I. Isolations and serological specificity. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **46**, 509-520 (1952).

22 Musso, D. & Gubler, D. J. Zika Virus. *Clin Microbiol Rev* **29**, 487-524, doi:10.1128/CMR.00072-15 (2016).

23 Rasmussen, S. A., Jamieson, D. J., Honein, M. A. & Petersen, L. R. Zika Virus and Birth Defects - Reviewing the Evidence for Causality. *N Engl J Med*, doi:10.1056/NEJMsr1604338 (2016).

24 Brasil, P. *et al.* Zika Virus Infection in Pregnant Women in Rio de Janeiro. *N Engl J Med* **375**, 2321-2334, doi:10.1056/NEJMoa1602412 (2016).

25 Cao-Lormeau, V. M. *et al.* Guillain-Barre Syndrome outbreak associated with Zika virus infection in French Polynesia: a case-control study. *Lancet* **387**, 1531-1539, doi:10.1016/S0140-6736(16)00562-6 (2016).

26 Enfissi, A., Codrington, J., Roosblad, J., Kazanji, M. & Rousset, D. Zika virus genome from the Americas. *Lancet* **387**, 227-228, doi:10.1016/S0140-6736(16)00003-9 (2016).

27 Petersen, L. R., Jamieson, D. J., Powers, A. M. & Honein, M. A. Zika Virus. *N Engl J Med*, doi:10.1056/NEJMra1602113 (2016).

28 Larocca, R. A. *et al.* Vaccine protection against Zika virus from Brazil. *Nature* **536**, 474-478, doi:10.1038/nature18952 (2016).

29 Dowd, K. A. *et al.* Rapid development of a DNA vaccine for Zika virus. *Science* **354**, 237-240, doi:10.1126/science.aai9137 (2016).

30 Abbink, P. *et al.* Protective efficacy of multiple vaccine platforms against Zika virus challenge in rhesus monkeys. *Science* **353**, 1129-1132, doi:10.1126/science.aah6157 (2016).

31 Richner, J. M. *et al.* Modified mRNA Vaccines Protect against Zika Virus Infection. *Cell*, doi:10.1016/j.cell.2017.02.017 (2017).

32 Pardi, N. *et al.* Zika virus protection by a single low-dose nucleoside-modified mRNA vaccination. *Nature*, doi:10.1038/nature21428 (2017).

33 Shan, C. *et al.* A live-attenuated Zika virus vaccine candidate induces sterilizing immunity in mouse models. *Nat Med*, doi:10.1038/nm.4322 (2017).

34 Xie, X. *et al.* Understanding Zika Virus Stability and Developing a Chimeric Vaccine through Functional Analysis. *MBio* **8**, doi:10.1128/mBio.02134-16 (2017).

35 Betancourt, D., de Queiroz, N. M., Xia, T., Ahn, J. & Barber, G. N. Cutting Edge: Innate Immune Augmenting Vesicular Stomatitis Virus Expressing Zika Virus Proteins Confers Protective Immunity. *J Immunol* **198**, 3023-3028, doi:10.4049/jimmunol.1602180 (2017).

36 Richner, J. *et al.* Vaccine mediated protection against Zika virus induced congenital disease. *Cell* **170**, 273–283 (2017).

37 Shan, C. *et al.* A single-dose live-attenuated vaccine prevents Zika virus pregnancy transmission and testis damage. *Nature Communications*, in press (2017).

38 Barouch, D. H., Thomas, S. J. & Michael, N. L. Prospects for a Zika Virus Vaccine. *Immunity* **46**, 176-182, doi:10.1016/j.immuni.2017.02.005 (2017).

39 Xie, X., Zou, J., Shan, C. & Shi, P. Y. Small Molecules and Antibodies for Zika Therapy. *J Infect Dis* **216**, S945-S950, doi:10.1093/infdis/jix406 (2017).

40 Lindenbach, B. D., Murray, C.L., Thiel, H.J., Rice, C.M. *Flaviviridae. In Fields Virology, 6th, Vol. 1, D.M. Knipe and P.M. Howley, eds.*, ((Philadelphia: Lippincott William & Wilkins), pp. 712–746, 2013).

41 Tricou, V. *et al.* A randomized controlled trial of chloroquine for the treatment of dengue in Vietnamese adults. *PLoS Negl Trop Dis* **4**, e785, doi:10.1371/journal.pntd.0000785 (2010).

42 Tam, D. T. *et al.* Effects of short-course oral corticosteroid therapy in early dengue infection in Vietnamese patients: a randomized, placebo-controlled trial. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* **55**, 1216-1224, doi:10.1093/cid/cis655 (2012).

43 Nguyen, N. M. *et al.* A randomized, double-blind placebo controlled trial of balapiravir, a polymerase inhibitor, in adult dengue patients. *J Infect Dis* **207**, 1442-1450, doi:10.1093/infdis/jis470 (2013).

44 Low, J. G. *et al.* Efficacy and safety of celgosivir in patients with dengue fever (CELADEN): a phase 1b, randomised, double-blind, placebo-controlled, proof-of-concept trial. *The Lancet. Infectious diseases* **14**, 706-715, doi:10.1016/S1473-3099(14)70730-3 (2014).

45 Whitehorn, J. *et al.* Lovastatin for adult patients with dengue: protocol for a randomised controlled trial. *Trials* **13**, 203, doi:10.1186/1745-6215-13-203 (2012).

46 Byrd, C. M. *et al.* A novel inhibitor of dengue virus replication that targets the capsid protein. *Antimicrob Agents Chemother* **57**, 15-25, doi:10.1128/AAC.01429-12 (2013).

47 Scaturro, P. *et al.* Characterization of the mode of action of a potent dengue virus capsid inhibitor. *J Virol* **88**, 11540-11555, doi:10.1128/JVI.01745-14 (2014).

48 Dokland, T. *et al.* West Nile virus core protein; tetramer structure and ribbon formation. *Structure* **12**, 1157-1163, doi:10.1016/j.str.2004.04.024 (2004).

49 Shang, Z., Song, H., Shi, Y., Qi, J. & Gao, G. F. Crystal Structure of the Capsid Protein from Zika Virus. *J Mol Biol*, doi:10.1016/j.jmb.2018.02.006 (2018).

50 Xie, X. *et al.* Inhibition of dengue virus by targeting viral NS4B protein. *J Virol* **85**, 11183-11195, doi:10.1128/JVI.05468-11 (2011).

51 Wang, Q. Y. *et al.* Discovery of Dengue Virus NS4B Inhibitors. *J Virol* **89**, 8233-8244, doi:10.1128/JVI.00855-15 (2015).

52 Niyomrattanakit, P. *et al.* A fluorescence-based alkaline phosphatase-coupled polymerase assay for identification of inhibitors of dengue virus RNA-dependent RNA polymerase. *J Biomol Screen* **16**, 201-210 (2010).

53 Zou, G., Xu, H. Y., Qing, M., Wang, Q. Y. & Shi, P. Y. Development and characterization of a stable luciferase dengue virus for high-throughput screening. *Antiviral Res* **91**, 11-19, doi:10.1016/j.antiviral.2011.05.001 (2011).

54 Abel, S., Back, D. J. & Vourvahis, M. Maraviroc: pharmacokinetics and drug interactions. *Antivir Ther* **14**, 607-618 (2009).

55 Shepherd, J., Waugh, N. & Hewitson, P. Combination therapy (interferon alfa and ribavirin) in the treatment of chronic hepatitis C: a rapid and systematic review. *Health Technol Assess* **4**, 1-67 (2000).

56 Cooksley, W. G. The role of interferon therapy in hepatitis B. *MedGenMed* **6**, 16 (2004).

57 Libraty, D. H. *et al.* Differing influences of virus burden and immune activation on disease severity in secondary dengue-3 virus infections. *J Infect Dis* **185**, 1213-1221, doi:10.1086/340365 (2002).

58 Libraty, D. H. *et al.* High circulating levels of the dengue virus nonstructural protein NS1 early in dengue illness correlate with the development of dengue hemorrhagic fever. *J Infect Dis* **186**, 1165-1168, doi:10.1086/343813 (2002).

59 Xie, X., Zou, J., Wang, Q. Y. & Shi, P. Y. Targeting dengue virus NS4B protein for drug discovery. *Antiviral Res* **118**, 39-45, doi:10.1016/j.antiviral.2015.03.007 (2015).

60 Guo, F. *et al.* A Novel Benzodiazepine Compound Inhibits Yellow Fever Virus Infection by Specifically Targeting NS4B Protein. *J Virol*, doi:10.1128/JVI.01253-16 (2016).

61 Zou, G. *et al.* Functional analysis of two cavities in flavivirus NS5 polymerase. *J Biol Chem* **286**, 14362-14372, doi:10.1074/jbc.M110.214189 (2011).

62 Yin, Z. *et al.* An adenosine nucleoside inhibitor of dengue virus. *Proc Natl Acad Sci U S A* **106**, 20435-20439, doi:10.1073/pnas.0907010106 (2009).

63 Schul, W., Liu, W., Xu, H. Y., Flamand, M. & Vasudevan, S. G. A dengue fever viremia model in mice shows reduction in viral replication and suppression of the inflammatory response after treatment with antiviral drugs. *J Infect Dis* **195**, 665-674 (2007).

64 Shan, C. *et al.* An Infectious cDNA Clone of Zika Virus to Study Viral Virulence, Mosquito Transmission, and Antiviral Inhibitors. *Cell Host Microbe* **19**, 891-900, doi:10.1016/j.chom.2016.05.004 (2016).

65 Gorman, M. J. *et al.* Host and genetic viral adaptations enable development of an immunocompetent mouse model of Zika virus infection. In revision (2018).

66 Zou, J. *et al.* Dimerization of flavivirus NS4B protein. *J Virol* **88**, 3379-3391, doi:10.1128/JVI.02782-13 (2014).

67 Youn, S. *et al.* Evidence for a Genetic and Physical Interaction between Nonstructural Proteins NS1 and NS4B That Modulates Replication of West Nile Virus. *Journal of virology* **86**, 7360-7371, doi:10.1128/jvi.00157-12 (2012).

68 Umareddy, I., Chao, A., Sampath, A., Gu, F. & Vasudevan, S. G. Dengue virus NS4B interacts with NS3 and dissociates it from single-stranded RNA. *J Gen.Virol.* **87**, 2605-2614, doi:87/9/2605 [pii];10.1099/vir.0.81844-0 [doi] (2006).

69 Zou, J. *et al.* Mapping the Interactions between the NS4B and NS3 Proteins of Dengue Virus. *Journal of virology* **89**, 3471-3483, doi:Doi 10.1128/Jvi.03454-14 (2015).

70 Zou, J. *et al.* Characterization of dengue virus NS4A and NS4B protein interaction. *J Virol* **89**, 3455-3470, doi:10.1128/JVI.03453-14 (2015).

71 Liang, Q. *et al.* Zika Virus NS4A and NS4B Proteins Deregulate Akt-mTOR Signaling in Human Fetal Neural Stem Cells to Inhibit Neurogenesis and Induce Autophagy. *Cell Stem Cell* **19**, 663-671, doi:10.1016/j.stem.2016.07.019 (2016).

72 Blazquez, A. B., Martin-Acebes, M. A. & Saiz, J. C. Amino acid substitutions in the non-structural proteins 4A or 4B modulate the induction of autophagy in West Nile virus infected cells independently of the activation of the unfolded protein response. *Front Microbiol* **5**, 797, doi:10.3389/fmicb.2014.00797 (2014).

73 Chatel-Chaix, L. *et al.* Dengue Virus Perturbs Mitochondrial Morphodynamics to Dampen Innate Immune Responses. *Cell Host Microbe* **20**, 342-356, doi:10.1016/j.chom.2016.07.008 (2016).

74 Xia, H. *et al.* An evolutionary NS1 mutation enhances Zika virus evasion of host interferon induction. *Nat. Comm.* **In press** (2018).

75 Xie, X. *et al.* Zika Virus Replicons for Drug Discovery. *EBioMedicine* **12**, 156-160, doi:10.1016/j.ebiom.2016.09.013 (2016).

76 Xie, X., Gayen, S., Kang, C., Yuan, Z. & Shi, P. Y. Membrane topology and function of dengue virus NS2A protein. *J Virol* **87**, 4609-4622, doi:10.1128/JVI.02424-12 (2013).

77 Deng, Y. Q. *et al.* Adenosine Analog NITD008 Is a Potent Inhibitor of Zika Virus. *Open Forum Infect Dis* **3**, ofw175, doi:10.1093/ofid/ofw175 (2016).

78 (b) (3) (A), J., Roe, K., Orillo, B., Shi, P. Y. & Verma, S. Combined treatment of adenosine nucleoside inhibitor NITD008 and histone deacetylase inhibitor vorinostat represents an immunotherapy strategy to ameliorate West Nile virus infection. *Antiviral Res* **122**, 39-45, doi:10.1016/j.antiviral.2015.07.008 (2015).

79 Sofia, M. J. Beyond sofosbuvir: what opportunity exists for a better nucleoside/nucleotide to treat hepatitis C? *Antiviral Res* **107**, 119-124, doi:10.1016/j.antiviral.2014.04.008 (2014).

80 Mandour, M., Vliegen, I., Paeshuyse, J. & Neyts, J. Rational design of antiviral drug combinations based on equipotency using HCV subgenomic replicon as an in vitro model. *Antiviral Res* **149**, 150-153, doi:10.1016/j.antiviral.2017.11.010 (2018).

81 Lohmann, V. *et al.* Replication of subgenomic hepatitis C virus RNAs in a hepatoma cell line. *Science* **285**, 110-113 (1999).

82 Blight, K. J., Kolykhalov, A. A. & Rice, C. M. Efficient initiation of HCV RNA replication in cell culture. *Science* **290**, 1972-1974 (2000).

83 Trung, D. T. *et al.* Liver involvement associated with dengue infection in adults in Vietnam. *Am J Trop Med Hyg* **83**, 774-780, doi:10.4269/ajtmh.2010.10-0090 (2010).

84 Wolfe, M. S. & Harry-O'kuru. A concise synthesis of 2'-C-methylribonucleodides. *Tetrahedron Letters* **36**, 7611-7614 (1995).

85 Stein, D. S. & Moore, K. H. Phosphorylation of nucleoside analog antiretrovirals: a review for clinicians. *Pharmacotherapy* **21**, 11-34 (2001).

86 Chen, Y. L. *et al.* Activation of PBMCs by dengue virus infection depotentiates Balapiravir. *J Virol*, doi:10.1128/JVI.02841-13 (2013).

87 Wang, Q. Y. *et al.* Inhibition of dengue virus through suppression of host pyrimidine biosynthesis. *J Virol* **85**, 6548-6556, doi:10.1128/JVI.02510-10 (2011).

88 Wang, Q. Y. *et al.* A translation inhibitor that suppresses dengue virus in vitro and in vivo. *Antimicrob Agents Chemother* **55**, 4072-4080, doi:10.1128/AAC.00620-11 (2011).

89 Xie, X., Zou, J., Puttikhunt, C., Yuan, Z. & Shi, P. Y. Two distinct sets of NS2A molecules are responsible for dengue virus RNA synthesis and virion assembly. *J Virol* **89**, 1298-1313, doi:10.1128/JVI.02882-14 (2015).

90 Arnold, J. J. *et al.* Sensitivity of mitochondrial transcription and resistance of RNA polymerase II dependent nuclear transcription to antiviral ribonucleosides. *PLoS Pathog* **8**, e1003030, doi:10.1371/journal.ppat.1003030 (2012).

91 Oliphant, T. *et al.* Development of a humanized monoclonal antibody with therapeutic potential against West Nile virus. *Nat. Med.* (2005).

92 Lazear, H. M. *et al.* Interferon-lambda restricts West Nile virus neuroinvasion by tightening the blood-brain barrier. *Science translational medicine* **7**, 284ra259, doi:10.1126/scitranslmed.aaa4304 (2015).

93 Zhang, S. *et al.* Development of resistance to passive therapy with a potently neutralizing humanized monoclonal antibody against West Nile virus. *J Infect Dis* **200**, 202-205, doi:10.1086/599794 (2009).

94 Engle, M. J. & (b) (3) (A), M. S. Antibody prophylaxis and therapy against West Nile virus infection in wild-type and immunodeficient mice. *J Virol* **77**, 12941-12949 (2003).

95 Li, B. *et al.* Butyrylcholinesterase, paraoxonase, and albumin esterase, but not carboxylesterase, are present in human plasma. *Biochem Pharmacol* **70**, 1673-1684, doi:10.1016/j.bcp.2005.09.002 (2005).

96 Sheahan, T. P. *et al.* Broad-spectrum antiviral GS-5734 inhibits both epidemic and zoonotic coronaviruses. *Science translational medicine* **9**, doi:10.1126/scitranslmed.aal3653 (2017).

97 Lazear, H. M. *et al.* A Mouse Model of Zika Virus Pathogenesis. *Cell Host Microbe* **19**, 720-730, doi:10.1016/j.chom.2016.03.010 (2016).

98 Lo, M. K., Shi, P. Y., Chen, Y. L., Flint, M. & Spiropoulou, C. F. In vitro antiviral activity of adenosine analog NITD008 against tick-borne flaviviruses. *Antiviral Res* **130**, 46-49, doi:10.1016/j.antiviral.2016.03.013 (2016).

99 Yeo, K. L. *et al.* Synergistic suppression of dengue virus replication using a combination of nucleoside analogs and nucleoside synthesis inhibitors. *Antimicrob Agents Chemother* **59**, 2086-2093, doi:10.1128/AAC.04779-14 (2015).

100 Chen, Y. L. *et al.* Inhibition of dengue virus by an ester prodrug of an adenosine analog. *Antimicrob Agents Chemother* **54**, 3255-3261, doi:10.1128/AAC.00397-10 (2010).
101 Chen, Y. L. *et al.* Inhibition of dengue virus RNA synthesis by an adenosine nucleoside. *Antimicrob Agents Chemother* **54**, 2932-2939 (2010).
102 Chen, Y. L., Yokokawa, F. & Shi, P. Y. The search for nucleoside/nucleotide analog inhibitors of dengue virus. *Antiviral Res* **122**, 12-19, doi:10.1016/j.antiviral.2015.07.010 (2015).
103 Lanier, R. *et al.* Development of CMX001 for the Treatment of Poxvirus Infections. *Viruses* **2**, 2740-2762, doi:10.3390/v2122740 (2010).
104 Lehar, J. *et al.* Synergistic drug combinations tend to improve therapeutically relevant selectivity. *Nat Biotechnol* **27**, 659-666, doi:10.1038/nbt.1549 (2009).
105 Meletiadis, J., Verweij, P. E., TeDorsthorst, D. T., Meis, J. F. & Mouton, J. W. Assessing in vitro combinations of antifungal drugs against yeasts and filamentous fungi: comparison of different drug interaction models. *Med Mycol* **43**, 133-152 (2005).
106 Geary, N. Understanding synergy. *Am J Physiol Endocrinol Metab* **304**, E237-253, doi:10.1152/ajpendo.00308.2012 (2013).
107 Aid, M. *et al.* Zika Virus Persistence in the Central Nervous System and Lymph Nodes of Rhesus Monkeys. *Cell* **169**, 610-620 e614, doi:10.1016/j.cell.2017.04.008 (2017).
108 Coffey, L. L. *et al.* Zika Virus Tissue and Blood Compartmentalization in Acute Infection of Rhesus Macaques. *PloS one* **12**, e0171148, doi:10.1371/journal.pone.0171148 (2017).
109 Dudley, D. M. *et al.* A rhesus macaque model of Asian-lineage Zika virus infection. *Nat Commun* **7**, 12204, doi:10.1038/ncomms12204 (2016).
110 (b) (3) (A) (b), A. J. *et al.* Zika Virus infection of rhesus macaques leads to viral persistence in multiple tissues. *PLoS Pathog* **13**, e1006219, doi:10.1371/journal.ppat.1006219 (2017).
111 Li, X. F. *et al.* Characterization of a 2016 Clinical Isolate of Zika Virus in Non-human Primates. *EBioMedicine* **12**, 170-177, doi:10.1016/j.ebiom.2016.09.022 (2016).
112 Osuna, C. E. *et al.* Zika viral dynamics and shedding in rhesus and cynomolgus macaques. *Nat Med* **22**, 1448-1455, doi:10.1038/nm.4206 (2016).
113 Adams Waldorf, K. M. *et al.* Fetal brain lesions after subcutaneous inoculation of Zika virus in a pregnant nonhuman primate. *Nat Med* **22**, 1256-1259, doi:10.1038/nm.4193 (2016).
114 (b) (3) (A) (b), A. J. *et al.* Zika virus infection in pregnant rhesus macaques causes placental dysfunction and immunopathology. *Nat Commun* **9**, 263, doi:10.1038/s41467-017-02499-9 (2018).

RESOURCE SHARING PLAN

Data sharing: Investigators in this proposed program recognize that promising methods, technologies, data and insights may arise during the course of their research. All investigators are aware of and agree to abide by the principles for sharing research resources, as described by NIH in “Principles and Guidelines for Recipients of NIH Research Grants and Contracts on Obtaining and Disseminating Biomedical Research Programs." The data generated in this grant will be presented at national or international conferences and published in a timely fashion. All final peer-reviewed manuscripts that arise from this proposal will be submitted to the digital archive PubMed Central. With regards to sequencing data, raw and processed sequencing datasets will be submitted to appropriate public databases (i.e., NCBI SRA and miRBase) following publications.

PLAN FOR SHARING MODEL ORGANISMS

Through the proposed research, we will generate recombinant flaviviruses with unique phenotypes *in vivo*. These recombinant viruses may be useful to other investigators who read our publications or hear about this information at scientific meetings. During our studies, we also anticipate the generation of reagents to study flavivirus replication including but not limited to antibodies, recombinant proteins, and expression vectors. If requests for these recombinant viruses and other reagents are received, we plan to provide these to the investigators after successful completion and approval of material transfer agreements.

AUTHENTICATION OF KEY BIOLOGICAL AND CHEMICAL RESOURCES

Cell lines. We have developed plans that will help us to avoid unreliable or misidentified resources. Cell lines used in our studies (BHK-21, C6/36, Vero, CHO, A549, and 293T cells) will be purchased from commercial vendors, such as (b) (3) (A), (b) (6), which confirm the authentication of cells. All cells used in culture will be tested routinely (several times per year) for the presence of mycoplasma.

Mice. All breeders of relevant mice are genotyped, phenotyped, and/or sequenced for accuracy. In addition, specific cells from mice are tested for the presence or absence of a given gene or protein using commercially available and validated antibodies or ones generated in the (b) (3) (A), (b) (6) laboratory. The congenic status of KO mice (e.g., *Ifnar1*$^{-/-}$ and *Ifnagr1*$^{-/-}$) or KI (hSTAT2) will be confirmed using detailed SNP panels that are available commercially (e.g., (b) (3) (A), (b) (6)). Other wild-type mice are purchased from commercial breeders.

Viruses. DENV-2, ZIKV, WNV, and YFV 17D will be generated from infectious cDNA clones. Recombinant reporter of DENV-2 and ZIKV will also be produced using infectious cDNA clones. Other flaviviruses, such as Powassan virus, other serotypes of DENV, and JEV will be obtained from Dr. Scott Weaver at World Reference Center for Emerging Viruses and Arboviruses at University of Texas Medical Branch. We will sequence our viral stocks, and any new stocks made from the original virus will be sequenced to ensure sequence stability for reproducibility. Plasmid DNA encoding WT and mutant viruses (DENV-2, WNV, or ZIKV) or structural genes will be sequenced in their entirety to confirm the presence or absence of introduced mutations (e.g., escape mutations). The genome encapsidated in viral particles of virus stocks also will be sequenced to confirm the presence or absence of defined mutations and to confirm that no other mutations have been introduced.

Antibodies. The flavivirus-specific mAbs used in these studies have been generated in the (b) (3) (A), (b) (6) laboratory. These antibodies have been validated by western blotting, ELISA assays against virions and purified virus proteins, neutralization assays, and flow cytometry of infected cells by the (b) (3) (A), (b) (6) laboratory. Additional antibodies will be purchased from reputable commercial vendors as needed and validated by western blotting. The specificity of commercial goat anti-mouse Ig detection reagents will be validated with detection of recombinant mouse monoclonal antibodies of defined isotype.

OMB Number: 4040-0001
Expiration Date: 10/31/2019

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

5. APPLICANT INFORMATION **Organizational DUNS*:** 0636907050000

Legal Name*: UNIVERSITY OF ALABAMA AT BIRMINGHAM
Department:
Division:
Street1*: UNIVERSITY OF ALABAMA AT BIRMINGHAM
Street2: 1720 2nd Ave South, (b) (3) (A), (b) (6)
City*: BIRMINGHAM
County:
State*: AL: Alabama
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 352940111

Person to be contacted on matters involving this application

Prefix:	First Name*:	Middle Name:	Last Name*:	Suffix:
	(b) (3) (A), (b) (6)		(b) (3) (A) (b)	

Position/Title:
Street1*: 1720 2nd Avenue South (b) (3) (A), (b) (6)
Street2:
City*: Birmingham
County:
State*: AL: Alabama
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 352940111
Phone Number* (b) (3) (A), (b) (6) Fax Number: (b) (3) (A), (b) (6) Email: (b) (3) (A), (b) (6)

7. TYPE OF APPLICANT* H: Public/State Controlled Institution of Higher Education

Other (Specify):
Small Business Organization Type ❍ Women Owned ❍ Socially and Economically Disadvantaged

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Project 4 - Influenza - UAB

12. PROPOSED PROJECT

Start Date*	Ending Date*
03/01/2019	02/29/2024

OMB Number: 4040-0010
Expiration Date: 10/31/2019

Project/Performance Site Location(s)

Project/Performance Site Primary Location

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	UNIVERSITY OF ALABAMA AT BIRMINGHAM
Duns Number:	0636907050000
Street1*:	UNIVERSITY OF ALABAMA AT BIRMINGHAM
Street2:	1720 2nd Ave South (b) (3) (A) (b) (6)
City*:	BIRMINGHAM
County:	
State*:	AL: Alabama
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	352940111
Project/Performance Site Congressional District*:	AL-007

Project/Performance Site Location 1

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	Southern Research Institute
DUNS Number:	0069005260000
Street1*:	2000 Ninth Ave. South
Street2:	
City*:	Birmingham
County:	
State*:	AL: Alabama
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	35205-5305
Project/Performance Site Congressional District*:	AL-007

Additional Location(s) File Name:

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ○ **Yes** ● **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ○ Yes ○ No

If YES, check appropriate exemption number: _ 1 _ 2 _ 3 _ 4 _ 5 _ 6 _ 7 _ 8

If NO, is the IRB review Pending? ○ **Yes** ○ **No**

IRB Approval Date:

Human Subject Assurance Number

2. Are Vertebrate Animals Used?* ● **Yes** ○ **No**

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ○ Yes ○ No

IACUC Approval Date:

Animal Welfare Assurance Number

3. Is proprietary/privileged information included in the application?* ● **Yes** ○ **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ○ **Yes** ● **No**

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ○ Yes ○ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ○ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ○ **Yes** ● **No**

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	Summary_Project_4.pdf
8. Project Narrative*	
9. Bibliography & References Cited	Project_4_final_Bibliography.pdf
10.Facilities & Other Resources	Project_4_Facilities_and_Resources.pdf
11.Equipment	Project_4_equipment.pdf
12. Other Attachments	Product_Development_Strategy.pdf

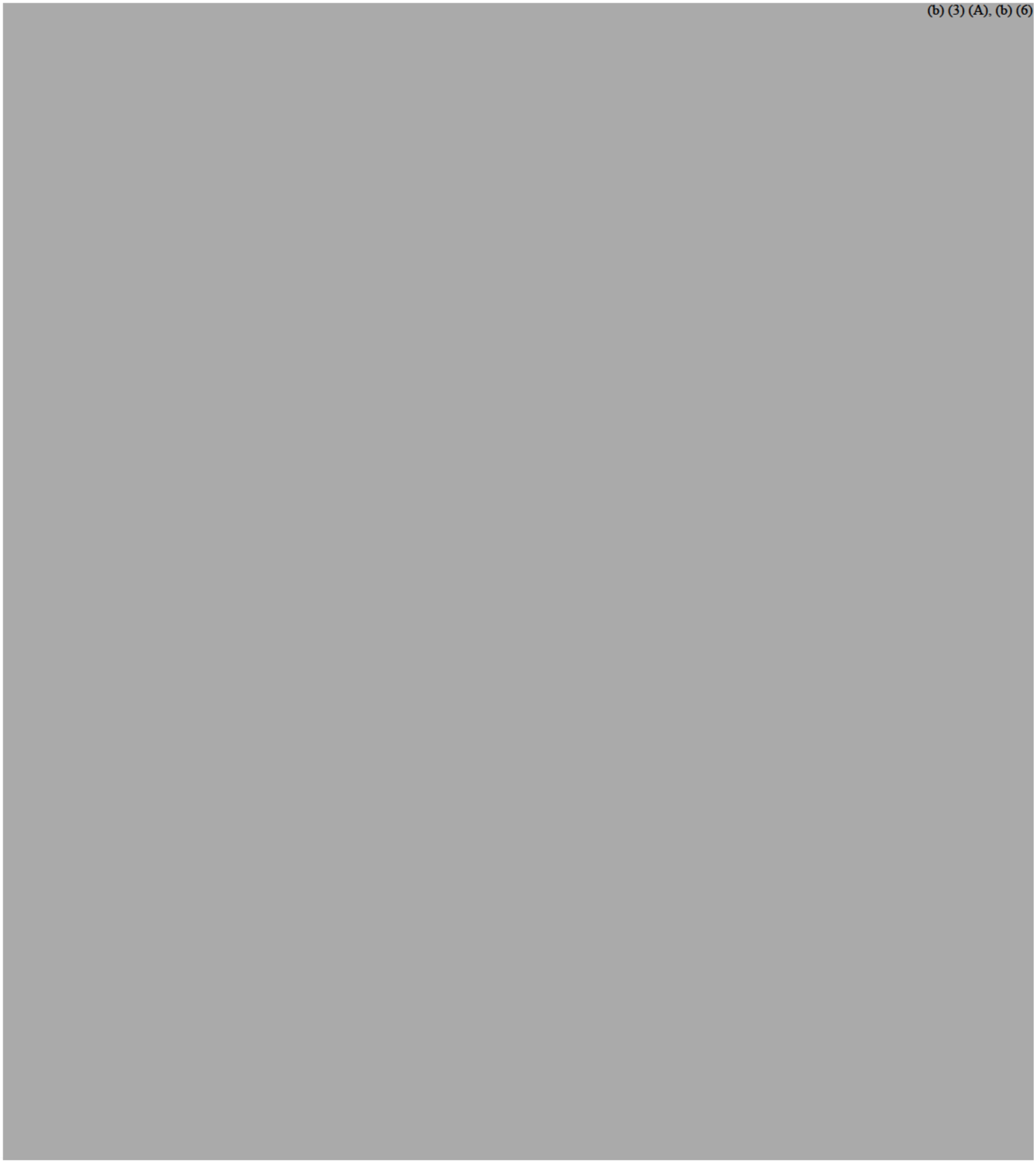
SUMMARY

The overall goal of this project is to identify new therapies that target influenza virus replication. The global health burden of annual influenza infections and periodic epidemics coupled with the emergence of avian influenza viruses, like H5NX and H7N9, highlight the urgent need for new effective treatments. A primary concern with the current drugs used to treat influenza in the U.S. is the development of resistance mutations that negate therapeutic benefit. Both published evidence and clinical experience suggest strongly that targeting the influenza virus RNA dependent RNA polymerase (RdRp) complex is a rational approach for antiviral therapy. This complex is responsible for many viral functions, including 5´ cap recognition, endonucleolytic cleavage, RNA synthesis, and polyadenylation providing multiple functional domains as targets for antiviral drug therapy and combination therapy. Agents can target the distinct functions and, theoretically, reduce the minimize development of resistance since resistance mutations would likely reduce the fidelity of the RdRp. One oral agent targeting the endonuclease domain, baloxavir, has shown efficacy in uncomplicated influenza and was recently approved in Japan for treating influenza in adults and children. A second oral agent targeting the 5´ cap binding domain, pimodivir, has shown antiviral activity in uncomplicated influenza and is advancing in clinical development. A third oral agent, favipiravir, targeting the polymerase activity has been approved in Japan for treating novel influenza strains not inhibited by neuraminidase inhibitors. We have recently identified several potent molecules through a collaborative public private partnership that inhibit RdRp functions. This research team provides the medicinal chemistry expertise, follow up assays, and in vivo experience to transform active hits into lead compounds and promises to yield new classes of highly active molecules that target the RdRp complex.

Project 4.1 UAB

Facilities and Other Resources:

(b) (3) (A), (b) (6)



QiagenEZ-1 Advanced XL nucleic acid extractor
The workstation, qPCR instruments, and microplate readers equipped with dedicated Dell computers with internet access and accompanying printers.

Standard equipment:
Sartorius analytical balance on a weigh table with a Misonix FE-2620 workstation
ThermoScientific Nanodrop Lite Spectrophotometer
Twelve, Sterilgard III Advance class 2 biosafety cabinets (SG 603)
Three, (ThermoScientific Forma Stericult CO_2 incubators (3310)
Three, Stericult 200 incubators
Three, Forma Scientific Water Jacketed incubators
Five, Niko SM7-1 inverted microscopes
Two, Olympus TL-3 stereomicroscopes
Olympus BX41 fluorescent microscope
Eight, ThermoForma PowerPlus -86˚C ULT Freezers (8583) with shelving systems
ThermoScientific Locator 4 Plus Liquid Nitrogen Tank (8207) with monitoring system
Thermolyne Locator Jr. Cryo Liquid Nitrogen Tank (CY50955) with monitoring system
Four, -20˚C freezers
Three, combination refrigerator freezers and a glass front refrigeration cabinet
Branson Sonifier 450 sonicator
Two, Isotemp heat blocks
Mettler Toledo Seven Easy pH meter (8603)
Two, Mettler Toledo balances (BD601)
Six, Eppendorf Centrifuges 5415D
Two, Refrigerated Eppendorf table top centrifuges (5810F) with standard and microplate rotors
Four, thermal cyclers for PCR.

Scientific Environment:
Other important resources include the active research environment in the Pediatrics Department at UAB. This dynamic and interactive Department includes efforts by (b) (3) (A), (b) (6) to understand the natural history of HCMV infections, clinical studies of antiviral compounds by Drs. Richard Whitley, and (b) (3) (A), (b) (6) as part of the Cooperative Antiviral Studies Group, studies on vaccines by (b) (3) (A), (b) (6), the detection of different HCMV subtypes by (b) (3) (A), (b) (6), the development of clinical resistance by (b) (3) (A), (b) (6), and studies in molecular virology in the laboratory of (b) (3) (A), (b) (6). This group of scientists and clinicians are focused on understanding the pathogenesis of viral infections and developing effective therapies and provides a fertile environment to identify potential new therapies, confirm their antiviral activity in vitro and in vivo, and initiate clinical trials to assess their efficacy.

Additional Resources: The UAB campus is located adjacent to the Southern Research (SR) campus which provides multiple opportunities for the proposed Project investigators to collaborate and interact on a regular basis. Telecommunication equipment and meeting space is also available at SR for proposed team meetings with the research director and project staff.

SR FACILITIES AND OTHER RESOURCES

I. Institution Overview

Southern Research (SR) is a not-for-profit research organization that has been in operation since 1941. SR employs approximately 500 research scientists, technical staff and support staff, and has a long-standing record of productivity in conducting governmental and industrial research grants and contracts. SR has multiple locations throughout the U.S., including Birmingham, Alabama (the main campus); Durham, North Carolina; Frederick, Maryland; and Houston, Texas.

Table of Contents for Facilities and Other Resources

I. Institution Overview
II. DDS
III. Infectious Diseases Department
  a. General
  b. Shared Facilities
IV. PI Facilities
V. Institutional Shared Facilities
  a. Animal Facilities
  b. Histology Facilities

II. Drug Discovery Division (DDS)

DDS laboratories and offices are primarily located on SR's main campus, which includes more than (b) (3) (A), (b) (6) of office and laboratory space. A number of core facilities are available for use by the faculty, including biosafety level (BSL) -1, -2 and -3 laboratories. A campus diagram (Figure 1) illustrates the buildings, which are located within close proximity to allow ease of communication between various disciplines, departments and staff.

Aligned with the NIST Risk Management Framework (RMF), the Information Technology Systems Support Group provides PC and network services to the staff of SR. Services include procuring, maintaining, and repairing all servers, network equipment, desktop and laptop computers, and printers; providing secure file, print, and email services, and supporting various applications for the administrative and technical staff. The Facilities and Maintenance Department provides maintenance and repairs for all SR facilities. The institution has excellent secretarial support services and lab maintenance services. SR's Human Resources, Contracts, Procurement and Accounting offices provide administrative support through their implied functions. The facilities described in this proposal are currently active and available, as needed, for the proposed research.

(b) (3) (A), (b) (6)

Literature searches for the development of this proposal and creation of manuscripts for dissemination of findings would not be possible without the support of a highly-developed library system. As an affiliate of the (b) (3) (A), (b) (6) SR scientists have reciprocal privileges at UAB libraries, and our staff scientists regularly make use of the Lister Hill Health Sciences Library and the Mervyn Sterne Library of UAB, located a few blocks away. SR researchers also have access to an extensive list of online journals under the subscription list of the Lister Hill Health Sciences Library, Alabama's largest biomedical library, inclusive of important chemical, biochemical, and biological journals. The Birmingham Public Library, as well as the libraries of Samford University and Birmingham Southern College, are also available to SR faculty. Complete access to the library resources, including use of photocopying facilities, are available through each of these libraries. In addition, full-time library personnel are available to research and retrieve

information through the facility to access online several electronic databases of publications and patents. All library information is available online and accessible from faculty and staff offices as well as from outside the university through the internet. Additionally, UAB Lister Hill Library has an interlibrary loan system that links us to multiple other universities both nationally and internationally.

III. Infectious Diseases Department

a. General

The Infectious Diseases Department maintains advanced, well-established research programs focused on seeking effective antiviral and antibacterial drugs and vaccines, in addition to elucidating novel disease-causing mechanisms for a diverse array of microbial pathogens. The Department is dedicated to identifying novel mechanisms, druggable targets, and strategies for prevention and treatment of both bacterial and viral infectious diseases throughout the world. The bacteriology program is focused on the study of the molecular pathogenesis of relevant fastidious, emerging, re-emerging, and multidrug-resistant bacteria to develop new treatments using novel approaches. The virology program focuses on viruses of global health significance, including Influenza, Ebola, and Flaviviruses such as Zika, concentrating on several critical areas including viral pathogenesis at the cellular and molecular levels, target identification and development of novel target-based assays, discovery of small molecule antivirals, and development of contemporary vaccines.

b. Shared Facilities

(b) (3) (A), (b) (6)

IV. PI Facilities

Laboratory: The laboratory space contains necessary equipment to conduct a wide array of studies involving tissue and cell culture, microbiology, biochemistry, molecular and cell biology, virology, and microscopy imaging for immunological, microbiological, molecular, biochemical, and cellular biological research. The (b) (3) (A), (b) (6) molecular biology laboratory is located (b) (3) (A), (b) (6). The lab consists of two main lab rooms of (b) (3) respectively, dedicated to bacterial work and a smaller (b) room dedicated to cell culture. Each one contains three working benches and one biosafety cabinet. Additionally, his lab has two offices. Our center has dedicated animal facilities in the Division of Drug Development with well-trained personnel.

Clinical: N/A

Animal: See Institutional Shared Facilities and Equipment (Section V.a.)

Computer: All associated offices are fully networked and equipped with multiple computers. Based on preference, a Dell PC or iMac computer, which along with multiple printers and scanners, are available to all staff. The PI has a Dell laptop computer with an Intel(R) Core(TM) i7-4610M CPU @ 3.00GHz, 3001 Mhz, 2 Cores, 4 Logical Processors. In addition, laboratory computers are used to interface with the Alpha Innotech FluorChem HD2 Imager, the BioRad IQ5 RT PCR system, MagPix Multiplex Instrument, and the Leica DMLB Epifluorescence microscope. All of the computers and printers are connected to the SR LAN, and all of the computers have access to the internet through this network. Computer core services use an Ethernet board network to printers and provide institutional file servers and extensive back-up capacities. The computers have ample storage space with which to enter, process, and analyze all data gathered from this project. In addition, assorted software is available for bioinformatics, data and statistical analysis informatics (e. g. SnapGene 3.2.1 and GraphPad Prism 7).

Office: Standard, secure offices are available for the PI and all staff, and are adjacent to the laboratory. There is additional office space for postdoctoral fellows, graduate students and technical support staff totaling (b) (3) (A) (b) not including DDS space for administrative and secretarial support staff.

V. Institutional Shared Facilities

a. Animal Facilities

SR has fully accredited AAALAC animal facilities. The Department of Laboratory Animal Resources (DLAR), Animal Care and Resources (ACR) and the Institutional Animal Care and Use Committee (IACUC) of SR provide the guide for the animal care and use program. Animal studies for various projects will be housed in a (b) (3) (A), (b) (6) animal facility, (b) (3) (A), (b) (6). This building is completely self-sufficient, is AAALAC accredited, and conforms to all applicable regulatory guidelines and requirements. (b) (3) (A), (b) (6) designed for small animals such as rodents and rabbits. These rooms are equipped with removable laboratory hoods for filling of feeders, gavage dosing, skin-painting, and other procedures requiring use of hoods. Two pairs of rooms have a nonstructural, temporary wall that can easily be removed to accommodate larger studies. This facility is designed as a dual-corridor barrier system with designated "clean" (*i.e.,* access) and "dirty" (*i.e.,* return) areas, as well as restricted-access areas. The rodent facility is also equipped with a clean/dirty necropsy suite. The rodent facility has a strict "access-return corridor" design with environmentally controlled directional air flow. (b) (3) (A), (b) (6) Critical support services, including teratology, andrology, dose formulation, necropsy, histology, veterinary care, and pathology, are located within or adjacent to the animal housing areas in the same facility. (b) (3) (A), (b) (6) Numerous features are incorporated into the design of this facility to provide personal safety and health protection of laboratory personnel, environmental protection, and a suitable environment for short- and long-term animal studies.

b. Histology Facilities

The histology laboratories occupy (b) (3) (A), (b) (6) (b) (3) (A). The histology laboratories include the following areas: slide review and quality control, staining and coverslipping, microtomy, embedding, tissue processing, and tissue trimming. Hooded work areas totaling approximately (b) (3) (A) (b) are located in the staining and cover-slipping and tissue trimming areas for personnel protection. All effluent air from the histology laboratories passes through medium efficiency filters prior to exhaust. The following equipment is available in this area: three automatic tissue processors, two embedding centers, ten rotary microtomes, one automatic staining machine, one automatic slide labeler, one automatic cassette labeler, one automatic coverslipper, three drying ovens, one solvent recycler, one formalin recycler, an ice maker, water baths, slide warmers, one refrigerator, one plastic bag sealing station, and eight microscopes (one per station).

Whitley Project 4.1
The following equipment is available in the [redacted] (b) (3) (A), (b) (6) laboratories that will be used for this project:

Equipment
Computer: All the major instrumentation including both BioMek 4000 autodilutors, both luminometers, and all the RealTime thermal cyclers have dedicated computers. Eight personal computers, and six laser jet printers are also present in the lab and are available for laboratory personnel.

Other:
Major equipment:

[redacted] (b) (3) (A), (b) (6)

Standard equipment:
Sartorius analytical balance on a weigh table with a Misonix FE-2620 workstation
ThermoScientific Nanodrop Lite Spectrophotometer
Twelve, Sterilgard III Advance class 2 biosafety cabinets (SG 603)
Three, (ThermoScientific Forma Stericult CO_2 incubators (3310)
Three, Stericult 200 incubators
Three, Forma Scientific Water Jacketed incubators
Five, Niko SM7-1 inverted microscopes
Two, Olympus TL-3 stereomicroscopes
Olympus BX41 fluorescent microscope
Eight, ThermoForma PowerPlus -86˚C ULT Freezers (8583) with shelving systems
ThermoScientific Locator 4 Plus Liquid Nitrogen Tank (8207) with monitoring system
Thermolyne Locator Jr. Cryo Liquid Nitrogen Tank (CY50955) with monitoring system
Four, -20˚C freezers
Three, combination refrigerator freezers and a glass front refrigeration cabinet
Branson Sonifier 450 sonicator
Two, Isotemp heat blocks
Mettler Toledo Seven Easy pH meter (8603)
Two, Mettler Toledo balances (BD601)
Six, Eppendorf Centrifuges 5415D
Two, Refrigerated Eppendorf table top centrifuges (5810F) with standard and microplate rotors
Four, thermal cyclers for PCR.

SR MAJOR EQUIPMENT

(b) (3) (A), (b) (6)

In addition to miscellaneous small equipment, larger and/or specialized equipment includes: multiple class II A/B biological safety cabinets, Milli-Q water purification system, gradient and non-gradient PCR thermocyclers, BioRad IQ5 RT PCR system, PCR clean room, sonic dismembrator, Qiagen TissueLyser-LT, Leica DMLB Epifluorescence microscope, Nikon SMZ800N Stereomicroscope, laser capture microdissection system, Invitrogen Countess Cell Counter, Alpha Innotech FluorChem HD2 Imager, GE 2-D gel electrophoresis system, BioRad I-Mark Microplate Reader, a BioTek 405_{LS} Microplate Washer, MagPix Multiplex Instrument, and an Eppendorf Biophotometer.

PRODUCT DEVELOPMENT STRATEGY

Milestones and Timeline

Introduction

As required by this RFA, outlined below are the key milestones and associated timelines for the Antiviral Drug Discovery and Development Center (AD3C) of which Dr. Richard Whitley is the Program Director. The theme is to *develop new small molecule therapeutics for emerging and re-emerging viral infections by inhibiting viral replication.* Activities will include additional compound evaluation to identify novel chemical scaffolds, evaluation of a set of focused nucleoside analogs, the application of basic virology research activities to identify and further probe the exact mechanism of action of identified lead molecules on viral replication. Medicinal chemistry and lead development will advance identified compounds through the drug discovery and development pathway, ultimately leading to preclinical evaluation of drug candidates to identify the most promising compounds for clinical evaluation. The AD3C has four interrelated research projects, with a focus on coronaviruses, alphaviruses, flaviruses and influenza. Their efforts will be supported by three cores: an Administrative Core (Core A), an Assay Core (Core B) and a Medicinal Chemistry and Lead Development Core (MCLDC; Core C). Organization and interaction between all projects and cores will be mediated out of the Administrative Core, with day to day AD3C operations led by the Core's personnel. Also, an Executive Committee (EC) consisting of all project and core leaders will regularly review the projects' and cores' activities and productivity. Finally, a Scientific Advisory Committee (SAC) will be established to annually provide a high-level evaluation of AD3C's progress and successes, and aid in refining the Center's activities and direction.

The four projects working with the Assay Core, Medicinal Chemistry and Lead Development Core and the Administrative Core will advance compounds through advanced testing, medicinal chemistry, lead optimization and *in vivo* efficacy studies. Each project will have the goal of identifying a lead compound as well as a backup compound for in vivo evaluation, ultimately leading to IND enabling studies. The overarching goal is to find broad spectrum mechanisms and compounds for the treatment of emerging pathogens. The AD3C is uniquely qualified to do so, based upon the strategies outlined in the proposals, the team of scientists assembled and the experience in collaborative drug discovery between Southern Research (SR) and the University of Alabama at Birmingham (UAB). The basic science and mechanistic studies will be conducted by the four Projects and the overall profile of what emerges will vary depending upon the results obtained. The three Cores will coordinate the development activities. The exact profile of the emerging lead compounds will vary and be science driven. However, to ensure that the AD3C can identify the compounds with the best overall profile and the greatest chance of succeeding, all the data will be available using the secure collaboration software ShareFile, to which all AD3C personnel has access.

Selection of an IND Candidate

The AD3C will utilize a pharmaceutical company model for discovery and development. In doing so we have established key attributes of a compound advancing to an IND candidate, which we will use as a guide in our preclinical studies. These include:

Synthesis

- No structural alerts or reactive groups
- No issues in scale up for GLP tox and Phase I studies No impurity issues
- No significant stereochemistry issues
- No obvious cost of goods issues

Biopharmaceutics

- Crystalline, MP >125, non-hygro
- Bioavailability and oral PK completed with crystalline compound
- Solubility, PK and dose compatible with standard immediate release formulation Stability profile compatible with delivery options
- Required delivery options defined and realistic

Absorption
- Conform with Rule of Five
- Predicted human intestinal absorption will be adequate to achieve predicted efficacious human exposure and fraction absorbed >30%
- Exposure in dog or monkeys using single PO dose attaining exposure equivalent to predicted clinical efficacious exposure

Clearance
- The predicted human PK for the compound should be assessed using in vitro parameters such as human liver microsome data and in vivo animal data
- Major CL mechanisms investigated in animals and predicted in humans including ID of likely CYPs and other metabolizing enzymes
- Variability in metabolic clearance assessed
- Projected human total CL < 10ml/min/kg
- Metabolic Drug-Drug Interaction (DDI)

PK/PD and Dose Prediction
- The in vivo relationship between drug concentration and pharmacodynamic response over time has been characterized and is well understood; the human clinical pharmacology predictions relating to dose (frequency and amount) and onset, duration and magnitude of response fall within the criteria defined in the product profile

Distribution
- Plasma or serum protein binding compared in human, pharmacology, and tox species; blood to plasma ratio determined

Pharmacology
- Target Identified
- Defined Mechanism of Action
- Human targets used for profiling
- If target is human, the potential impact of target polymorphisms has been considered.
- Potency differences between human target and species used in efficacy models is understood to guide human dose predictions
- Greater than 100x selectivity vs. relevant human targets

Safety
- Ames – negative in vitro test for bacterial mutagenicity
- Appropriate preclinical tox species selected
- Broad ligand profiling completed using standardized CEREP panel
- <50% inhibition of HERG, Nav1.5, LQT1, and L-type current at 100x the projected efficacious free-plasma levels in humans.

Intellectual Property
- Appropriate patent protection in place for the identified lead and back up compounds

These parameters will assist the AD3C throughout the project. In addition, for this project we have broken the drug discovery process up into key measurable milestones and placed the amount of time that will be needed to accomplish each (Figure 1). These timelines were established based upon past experience and industry standards and are set to identify an IND candidate. However, a large portion of the funds needed for the late stage preclinical and IND enabling GLP studies are not available as part of this RFA and additional funds will be sought.

Key Milestones

1. Identify lead compound series [EC_{90} < 1 μM; CC_{50} > 30 μM; VTR > 3 logs at 10 μM, MLM t½ >60 min; Sol. >10 μM]; advanced to spectrum of activity, potency and pharmacokinetic studies (18 months).
2. In vivo efficacy in mice for lead compound series [bioavailability F > 30%, half-life > 2 h]. (18-24 months).
3. Back up series of compounds with [EC_{50} (H3N2) < 0.5 μM, CC_{50} > 50 μM, and VLR > 3 logs]; evaluated for broad spectrum activity in different subtypes of influenza, including HPAI in *in vitro* assays (24- 42 months)
4. Characterize spectrum of antiviral activity and mechanism of action of lead compounds and backup series, and generate resistant isolates (36-60 months)
5. In vivo efficacy in HPAI [for compounds active against H1N1 infection in vivo] (36-60 months)
6. Pivotal efficacy studies in ferrets (48-60 months)

Each of these milestones has a number of associated studies and decision points that are listed below and outlined in Figure 1.

1) Lead compound series - identified (18 months) – 2 compounds
   a. Potency of EC_{50} (H3N2) < 1 μM, CC_{50} > 50 μM; Efficacy VTR > 3 logs at 10 μM against viral replication.
   b. In vitro SI >50.
   c. Tractable synthesis
   d. Chemical novelty- initial patent analysis
2) In vivo efficacy tested (18 -24 months) – 2 compounds
   a. Oral bioavailability of F >30% in mice, half- life >2h
   a. Bioavailability such that plasma concentration are 5-10 fold above EC_{50}
   b. Oral bioavailability of >20% in rodents preferred
   c. No inhibition of cytochrome P450's 10 μM (3A4, 2C9,2C19)
   d. Synthetic feasibility determined (availability of starting materials, number of steps, time lines to produce 25 -50 grams) and freedom to operate analysis completed.
3) Backup series of compounds (24-42 months) – 3 compounds
   a. Potency, EC_{50} (H3N2) < 0.5 μM, CC_{50} > 50 μM, SI > 50, and VLR > 3 logs];
   b. Tractable synthesis
   c. Structure-activity relationships versus a primary endpoint indicating antiviral activity or mechanism
   d. Chemical novelty- initial patent analysis
   e. Different scaffold from primary lead series
4) Spectrum of antiviral activity, mechanism of action of lead and backup compounds (12-48 months)
   a. Activity <10 μM against H1N1, H3N2, B, and HPAI strains
   b. Characterize function of RdRp inhibited by compound
   c. Generation of resistant isolates and sequencing to identify molecular determinants of resistance
   d. Characterize replicative fitness of resistant isolates and check cross resistance
5) In vivo efficacy in HPAI (36-60 months)
   a. Compounds must be active in mouse model of H1N1 infection
   b. No observable in vivo side-effects. CNS exposure if relevant (ED_{50} of <100 mg/Kg)
6) Pivotal efficacy studies in ferrets (36-60 months)
   a. A 7-day non-GLP toxicity study in rodents (Determination of No Effect Level (NOEL) and/or No Adverse Effect Level (NOAEL))
   b. Expanded PK studies including a second species (preferably dog). A plasma half-life of >2 hr (mouse or rat) and >30% bioavailability). Metabolite identification in vivo
   c. Understanding the metabolic pathways involved using rodent, dog and human microsomes

and hepatocytes as well as in vivo in rodents and dogs prior to toxicology studies.

d. Chemistry feasibility and cost of goods analysis, which will be key as larger quantities of compound (100-200 grams) would be needed for some of the preliminary studies. Stability studies.
e. Testing in a broad receptor profile including a set of key cardiac and CNS ion channels and receptors (no activity at 10μM).
f. Compound stability as a dry powder and in solution at a range of pH's

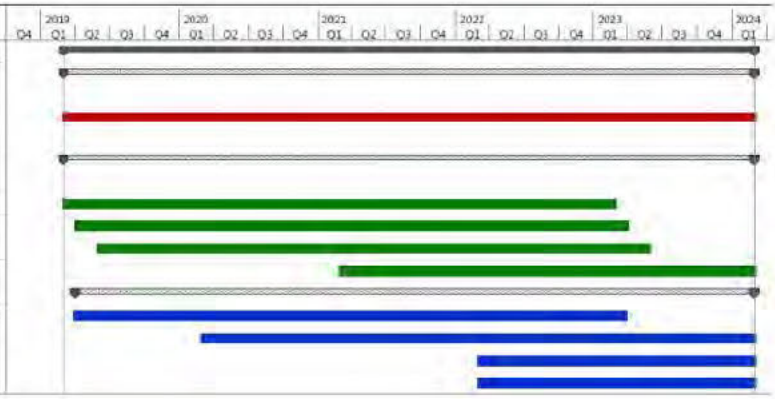
IND Enabling Studies (48-60 months)

- Scale -up of drug substance
- Stability studies to support tox
- Formulation development for tox studies
- 14-day toxicity study in rodents and dogs
- Cardiovascular assessment
- GMP Manufacture of 1Kg of drug substance
- Formulation development
- 14-day GLP toxicity study in rodents and dogs
- Cardiovascular assessment

These timelines are estimates and can vary depending upon compounds availability, resources and capacity at the GMP manufacturing facilities. The majority of these studies will be conducted with Southern Research's GLP facilities.

Figure 1 Timeline Influenza Project

ID	Task Name	Start	Finish
1	**Project 4**	**Fri 3/1/19**	**Thu 2/29/24**
2	**Aim 1. Identify Potent Influenza Compounds Targeting RdRp Complex Functions**	**Fri 3/1/19**	**Thu 2/29/24**
3	Aim 1. Identify Potent Influenza Compounds Targeting RdRp Complex Functions	Fri 3/1/19	Thu 2/29/24
4	**Aim 2. Define Mechanism of Action and Antiviral Spectrum**	**Fri 3/1/19**	**Thu 2/29/24**
5	Aim 2a. Define Spectrum of Antiviral Activity	Fri 3/1/19	Tue 2/28/23
6	Aim 2b. Confirm RdRp Function Inhibition	Wed 4/3/19	Fri 3/31/23
7	Aim 2c. Map Molecular Determinants of Resistance	Sat 6/1/19	Tue 5/30/23
8	Aim 2d. Combination Efficacy Studies	Mon 3/1/21	Thu 2/29/24
9	**Aim 3. In Vivo Evaluation & Bioavailability Studies**	**Mon 4/1/19**	**Thu 2/29/24**
10	Aim 3a. Pharmacokinetics & Bioavailability Studies	Mon 4/1/19	Wed 3/29/23
11	Aim 3b. Efficacy Testing in H1N1 Mouse Model	Sun 3/1/20	Thu 2/29/24
12	Aim 3b. Efficacy Testing in H5N1 Mouse Model	Tue 3/1/22	Thu 2/29/24
13	Aim 3c. Efficacy Testing in H1N1 Ferret Model	Tue 3/1/22	Thu 2/29/24



Product Development Plan

Introduction

The overall aim of the project is to identify novel therapies for the treatment of influenza infection. To do so, a series of go/no go decisions have been established with the goal of obtaining (an) orally active drug(s). Described below are the criteria required to identify lead compounds suitable for animal models testing, the selection of a preclinical compound with the efficacy and safety profile to advance into non-GLP studies, additional metabolic profiling and pharmacokinetic studies. Synthetic feasibility will also be considered. These studies will enable the team to select a compound for IND enabling GLP studies leading to a phase I clinical candidate(s).

1. Intended use/indication of the proposed product and the biodefense/public health gap the product is intended to fill

The global health burden of annual occurrence of influenza and periodic epidemics coupled with the emergence of highly pathogenic avian influenza (HPAI) has highlighted as urgent need for new effective treatments. A primary concern with the current influenza therapies (M2 ion channel and neuraminidase inhibitors) is the development of resistance mutations that can negate therapeutic benefit. Both published evidence and clinical experience suggest strongly that targeting the influenza virus RNA dependent RNA polymerase (RdRp) is a rational approach for antiviral therapy.

The RdRp is responsible for a number of viral functions, including 5´ cap recognition, endonucleolytic cleavage, RNA synthesis, and polyadenylation. Clearly, the heterotrimeric RdRp complex provides multiple functional domains that could be targets for antiviral drug therapy and might reduce the frequency of escape mutations to highly conserved domains. We have recently identified several active molecules from the Emory Institute for Drug Discovery (EIDD), Gilead Sciences (GS), and Southern Research (SR). This application provides all the necessary expertise in medicinal chemistry, follow up assays, and in vivo experience to transform active hits into lead compounds and advance them rapidly into animal models of influenza virus infection. The public private partnership with EIDD and GS leverages the proposed developmental program and promises to yield highly active molecules.

Three compounds targeting three distinct RdRp functions are in advanced stages of development in the US. These include the RdRp polymerase inhibitor, favipiravir (T-705), the PB2 cap binding inhibitor JNJ63623872 (VX-787), and the PA endonuclease inhibitor, S-033188 (baloxavir) (reviewed in (1, 2)). The polymerase inhibitor, T-705, has been approved in Japan and continues to undergo clinical testing in the US. The endonuclease inhibitor, baloxavir, was very recently approved in Japan and it was shown to reduce viral titers and levels of RNA significantly faster than oseltamivir, but the time to the cessation of all symptoms was not superior (3). Importantly, early clinical results suggest that resistance to this compound may emerge rapidly, namely ~9% among adults. The cap binding inhibitor, VX-787, is currently in Phase 2 clinical trials. Together, these drugs highlight not only the multiple functions of the RdRp, but also the critical nature of these functions in the viral cycle as well as their ability to be targeted with effective antiviral therapies. To date, none of these compounds have been approved by the US FDA but they highlight the promise of targeting the RdRp.

We intend to use the existing platforms that have been already been developed at Southern Research (SR) and focus on different sites of inhibition of polymerase. Our intent is to discover molecules that are active at different sites and, therefore, offer the opportunity for combination and broad spectrum activity.

2. Statement of the value of the project, including lay description of key technology objectives, innovation and advantages compared to competing products, technologies or services

The value of this project is the contribution of drugs to treat a broad group of Influenza viruses. In essence the value of the project resides in the multidisciplinary approach of a team of scientists with expertise in molecular biology, drug discovery, medicinal chemistry, animal models, and preclinical compound evaluation

and, ultimately, the translation of our molecule discovery program into clinical development if the opportunity presents. The key technological objectives are the application of a seamless approach to rapid drug development. Lead molecules for the inhibition of influenza replication have been identified that provide a technological starting point for product development. While other groups may pursue the discovery of lead molecules, such as those noted above, we have a distinct advantage of the availability of world class virology and medicinal chemistry being an integral component of our application.

3. Description of the goal(s) of the project, including one (or more) intermediate products, final product(s) or stage(s) of product development to be completed during the award period. (no specific product profile by licensing indication is needed)

As noted above, the primary goal of the project is to develop drugs to treat influenza. We already have established leads and assays and access to diverse compounds with activity and have molecules that can be used to initiate mechanism of action studies. Our goal is to identify additional lead molecules and improve the spectrum of activity and efficacy of these products. In addition, mechanistic studies will be performed. A detailed research plan has been established with clear criteria for compound advancement. Using a go/no go decision tree plan (see key milestones section) we will finalize two or three molecules that will provide a choice for a preclinical development pathway, leading to the selection of one or more clinical candidates, including the potential for combination.

4. A detailed description of the qualitative and quantitative criteria and data elements to be used to assess the scientific merit of feasibility of proceeding to the next stage of development

Most lead optimization programs produce multiple lead compounds, typically two to four, and require an additional series of pre-GLP studies to select the compound with the best profile. The decision tree to select the drug candidate will be based upon the collection of properties of the compounds including efficacy, safety profile, physical properties (stability, solubility, crystal forms, etc.), pharmacokinetics, ease and cost of synthesis, dosage and formulation (see key milestone section).

In parallel we will perform:

- Testing in a broad receptor profile including a set of key cardiac and CNS ion channels and receptors
- Compound stability as a dry powder and in solution at a range of pH's
- Examining the compound's effect on cytochrome P450's will be required as some of these compounds will be used in combination with other drugs
- Expanded PK studies including a second species (preferably dog, for which SR has the capability)
- Metabolite identification in vivo
- Chemistry feasibility and cost of goods analysis, which will be key as larger quantities of compound (100-200 grams) would be needed for some of the preliminary studies
- A 7-day non-GLP toxicity study to determine a maximum tolerated dose, identify any toxicities and establish doses for the GLP toxicity study (Determination of No Effect Level (NOEL) and/or No Adverse Effect Level (NOAEL))

5. A description of the distinct stages/activities that are gates for Go/No Go decisions for advancement

The active molecules in this application were derived from novel and diverse compound collections and were identified by assays designed to enrich hits with inhibitors that target RdRp function. The replication of influenza is a complex process that presents many opportunities for the development of novel antiviral therapies (4). Following infection, the viral ribonucleoproteins (vRNPs) are transported to the host cell nucleus. The heterotrimeric RdRp complex (PB1, PB2 and PA) together with the nucleoprotein (NP) then begins to initiate the replication process (5, 6). The synthesis of viral RNA does not require a primer, but rather, the viral RdRp initiates RNA synthesis internally on viral RNA. The RdRp can also direct mRNA synthesis through a process called cap snatching (reviewed in (6)). This process initiates when the RdRp is in a pre-initiation state with the PB2 cap-binding and PA endonuclease domains in alignment, such that a host capped RNA can be bound and cleaved about 13 nucleotides from the cap site. The cap binding domain then rotates to present the 3´ terminus to the

polymerase active site, and polyadenylation occurs by a stuttering mechanism. The multiple conformations of the heterotrimeric RdRp that direct many different functions including 5´ cap-recognition and endonuclease activity, transcription and polyadenylation of viral mRNAs, protein-protein interactions, and synthesis of + strand cRNA and - strand genomic vRNA. Active compounds presented here have been shown to inhibit at least two of these essential processes.

Hit compounds from collaborations with EIDD and GS. Existing collaborative studies with EIDD and GS evaluated small numbers of targeted molecules with a high probability of inhibiting the replication of influenza virus. EIDD has assembled a library of approximately 1000 nucleoside and nucleotide analogs, and from these, a subset of 25 coded compounds was evaluated in a series of CPE-based assays in MDCK cells with H1N1, H3N2, and B strains of influenza virus. One of the compounds, EIDD 1931, inhibited the replication of the HN1 strain with a potency of less than 1 μM (**Fig. 1**) and also inhibited an H3N2 and a B strains with EC_{50} values of 3.5 and 0.36 μM, respectively. This compound has also been reported to exhibit broad spectrum antiviral activity against many viruses, including Venezuelan equine encephalitis virus (VEEV), coronaviruses, chikungunya virus and hepatitis C virus (7). The mechanism of action of EIDD 1931 against VEEV is mediated through nsP4 (see Project 2), a member of the replication complex, and leads to the accumulation of mutations in viral RNA. This compound likely inhibits influenza virus in a similar manner and is predicted to inhibit the virus by inducing mutations as that reported for T-705 (8). A total of six additional compounds exhibited EC_{50} values of <10 μM against at least one virus. Similarly, a set of 12 coded compounds was obtained from GS and evaluated against a nanoluc reporter virus (A/California/04/2009 pdm09 (H1N1) PASTN) (9), in primary human small airway epithelial cells (Lonza, Basel, Switzerland). This approach identified four nucleosides and two nonnucleosides with EC_{50} values of <10 μM. Interestingly, four of the active non-nucleosides were structurally similar to GS 490260 (**Fig. 1**), which was reported previously to inhibit PA endonuclease activity by chelating metal ions in the amino terminus of the enzyme (10).

Data obtained in research studies will be continually reviewed in biweekly meetings as well as quarterly meetings of the Executive Committee will review ongoing data. At least yearly, the Scientific Advisory Committee will meet to review the drug development plan. The NIH project officer for this grant will be invited to attend the yearly meetings to guide us regarding NIH partnerships that could facilitate product development. As we approach the identification of a clinical candidate, the External Scientific Advisory Board will be able to assist the decision-making process.

From these targeted meetings, molecules will be selected for further development. Once a preclinical candidate is identified it would be tested in a fairly standard GLP program to collect the appropriate data for an IND. Parallel with this would be further optimization to provide sufficient API for phase I/II clinical studies. Depending on the route of administration (oral delivery is preferred), formulation and stability studies would be conducted to optimize these routes (oral or IV, other options). Ideally, the compound will have the appropriate bioavailability and half-life for oral administration. Therefore, the Phase I studies will be conducted with a powder in a bottle formulation; SR has experience using this approach for first in human testing. The powder-in-bottle (PIB) approach will be used to accelerate development and introduction into Phase I clinical trials. This approach consists of extemporaneously compounding the active pharmaceutical ingredient (API) into a solution or a suspension in the clinic prior to oral administration. The development will consist of physico-chemical assessment, constitution fluid selection, weighing and dosing validation, and stability evaluation of API, before and after constitution with the fluid. This approach will provide a high degree of dosing flexibility during the initial phase of clinical trials. Additionally, the PIB approach reduced the time and API required for clinical development and supplies to < 2 months and < 100 gm, respectively.

A general GLP toxicity package would be developed. Full Ames and Mouse Lymphoma genotoxicity studies would be conducted. This would include acute oral and intravenous studies in rat and/or mouse and another species, typically dog for typically 28 days. The choice of species will depend in part upon the efficacy animal models. The length of the studies will be determined based upon the phase I/II clinical plan. A GLP safety pharmacology package would be generated that could include rat, dog (or primates depending on species selections above), Erwin studies and cardiovascular safety studies in 2 species and Perkinje Fiber electrophysiology. After IND filing, long term genotoxicity studies as well as longer term animal safety studies would be initiated. Additional funding for these studies would be required.

In summary, a preclinical compound would be orally active in a mouse model with dosing of ≤10 mg/kg and based upon the in vitro activity and pharmacokinetic profile the projected human dose would be <500 mg/day. A compound meeting these criteria from any of the projects would be designated a preclinical candidate and thus advance to a series of FDA regulated GLP studies required for IND submission.

The key milestones for the GLP studies and IND submission include:

1) GMP manufacturing of 500 -1000 grams of API to be used for GLP toxicity studies and formulation development. On average this can take approximately 6 months.
2) Identification of a second species for GLP toxicity studies. Typically dogs, but non-human primates may offer some advantages for certain indications. Once initiated the GLP toxicity studies will take about 6-9 months to complete the in vivo studies and generate the reports.
3) Preparation of the IND

The performance specs/features the product should have in order to provide therapeutic benefit

- Small molecule (MW <500), plasma half-life of >2 hr (mouse or rat) and >30% bioavailability
- In vitro antiviral activity and selectivity versus normal cells. Ranges for antiviral potency would be typically < 0.1μM and > 20 μM versus normal cells.
- Primary endpoint indicating antiviral activity or mechanism
- Oral activity in a relevant mouse and ferret model of infection
- Twice a day administration at most
- 5-14 day treatment course
- Absence of bone marrow suppression or hepatic toxicity.
- No observable in vivo side-effects.
- Understanding the metabolic pathways involved using both rodent, dog and human microsomes as well as in vivo in rodents and dogs prior to toxicology studies.
- Tissue distribution
- Synthetic feasibility determined (availability of starting materials, number of steps, time lines to produce 25 -50 grams) and freedom to operate analysis completed
- Absence of development limiting adverse events (seizures in an animal model)
- Tissue distribution to target organs
- No inhibition of cytochrome 3A4 or 2C9 at 10 μM

6. A description and developmental status of the assays for the product release and characterization, including activity and efficacy (this may not be needed per se; depends on the stage of drug development)

After the mouse studies have been completed and lead compounds will be evaluated in a ferret model. The ferret model is the “gold standard” for testing therapeutics and vaccines against influenza strains as these animals are readily infected with human strains and follow a similar disease progression as humans (11).

7. Discussion with the FDA, if any, relevant to the development activities of the candidate product.

We envision involving the FDA on an informal basis (if allowed) in order to expedite drug development for treatment of influenza. Current FDA recommendations for hospital-based studies have not proven feasible. This has worked most successfully for the PI (Richard Whitley) in studies performed by the NIAID Collaborative Antiviral Study Group. To be very specific, the NIAID CASG has had joint discussions with the Antiviral Division of the FDA involving unmet medical need and the proper clinical pathways to meet these needs. Toward this end, the Antiviral Branch of the FDA may have very specific issues that we will need to address in this application.

REFERENCES

1. Koszalka P, Tilmanis D, Hurt AC. Influenza antivirals currently in late-phase clinical trial. Influenza Other Respir Viruses. 2017;11(3):240-6. PubMed PMID: 28146320; PubMed Central PMCID: PMCPMC5410715.
2. Naesens L, Stevaert A, Vanderlinden E. Antiviral therapies on the horizon for influenza. Curr Opin Pharmacol. 2016;30:106-15. PubMed PMID: 27570127.
3. Portsmouth S, Kawaguchi K, Mastugu A, Tsuchiya K, Uehara T, editors. Cap-dependent Endonuclease Inhibitor S-033188 for the Treatment of Influenza: Results from a Phase 3, Randomized, Double-Blind, Placebo- and Active Controlled Study in Otherwise Healthy Adolescents and Adults with Seasonal Influenza. IDWeek 2017; 2017; San Diego, CA: Oxford University Press.
4. Shim JM, Kim J, Tenson T, Min JY, Kainov DE. Influenza Virus Infection, Interferon Response, Viral Counter-Response, and Apoptosis. Viruses. 2017;9(8). PubMed PMID: 28805681; PubMed Central PMCID: PMCPMC5580480.
5. Nayak DP, Balogun RA, Yamada H, Zhou ZH, Barman S. Influenza virus morphogenesis and budding. Virus Res. 2009;143(2):147-61. PubMed PMID: 19481124; PubMed Central PMCID: PMCPMC2730999.
6. Te Velthuis AJ, Fodor E. Influenza virus RNA polymerase: insights into the mechanisms of viral RNA synthesis. Nat Rev Microbiol. 2016;14(8):479-93. PubMed PMID: 27396566; PubMed Central PMCID: PMCPMC4966622.
7. Urakova N, Kuznetsova V, Crossman DK, Sokratian A, Guthrie DB, Kolykhalov AA, et al. beta-D-N(4)-hydroxycytidine is a potent anti-alphavirus compound that induces high level of mutations in viral genome. J Virol. 2017. PubMed PMID: 29167335; PubMed Central PMCID: PMCPMC5774879.
8. Bank C, Renzette N, Liu P, Matuszewski S, Shim H, Foll M, et al. An experimental evaluation of drug-induced mutational meltdown as an antiviral treatment strategy. Evolution. 2016;70(11):2470-84. PubMed PMID: 27566611.
9. Tran V, Moser LA, Poole DS, Mehle A. Highly sensitive real-time in vivo imaging of an influenza reporter virus reveals dynamics of replication and spread. J Virol. 2013;87(24):13321-9. PubMed PMID: 24089552; PubMed Central PMCID: PMCPMC3838222.
10. Hastings JC, Selnick H, Wolanski B, Tomassini JE. Anti-influenza virus activities of 4-substituted 2,4-dioxobutanoic acid inhibitors. Antimicrob Agents Chemother. 1996;40(5):1304-7. PubMed PMID: 8723491; PubMed Central PMCID: PMCPMC163316.
11. Oh DY, Hurt AC. Using the Ferret as an Animal Model for Investigating Influenza Antiviral Effectiveness. Front Microbiol. 2016;7:80. PubMed PMID: 26870031; PubMed Central PMCID: PMCPMC4740393.

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: First Name*: RICHARD Middle Name J. Last Name*: WHITLEY Suffix:

Position/Title*: Distinguished Professor
Organization Name*: University of Alabama at Birmingham
Department: Pediatrics
Division:
Street1*: (b) (3) (A), (b) (6)
Street2:
City*: Birmingham
County:
State*: AL: Alabama
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 352331711

Phone Number*: 205-934-5316 Fax Number: 205-934-8559

E-Mail*: rwhitley@peds.uab.edu

Credential, e.g., agency login: (b) (6)

Project Role*: Other (Specify) Other Project Role Category: Project Lead

Degree Type: MD Degree Year: 1971

Attach Biographical Sketch*: File Name:

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: First Name*: MARK Middle Name Neal Last Name*: PRICHARD Suffix:

Position/Title*: Professor
Organization Name*: University of Alabama at Birmingham
Department:
Division:
Street1*: (b) (3) (A), (b) (6)
Street2:
City*: BIRMINGHAM
County:
State*: AL: Alabama
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 352331711

Phone Number*: (205) 934-1990 Fax Number: (205) 975-1992

E-Mail*: MPRICHARD@PEDS.UAB.EDU

Credential, e.g., agency login: (b) (6)

Project Role*: Co-Investigator Other Project Role Category:

Degree Type: Ph.D. Degree Year: 1992

Attach Biographical Sketch*: File Name: Prichard_Biosketch_influenza_2018-complete.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: (b) (3) (A), (b) Middle Name Last Name*: (b) (3) (A), (b) (6) Suffix:

Position/Title*: Assistant Fellow
Organization Name*: Southern Research
Department: Infectious Diseases
Division: Drug Discovery
Street1*: 2000 9th Ave S
Street2:
City*: Birmingham
County:
State*: AL: Alabama
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 35205-3505

Phone Number*: (b) (3) (A), (b) (6) Fax Number:

E-Mail*: (b) (3) (A), (b) (6)

Credential, e.g., agency login: (b) (6)

Project Role*: Other (Specify) Other Project Role Category: Project Co-lead

Degree Type: Ph.D. Degree Year: (b) (3) (A)

Attach Biographical Sketch*: File Name: Biosketch_(b)(3).pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: (b) (3) (A), (b) (6) Middle Name (b) (3) Last Name*: (b) (3) (A), (b) (6) Suffix:

Position/Title*:	Senior Project Manager
Organization Name*:	Southern Research
Department:	
Division:	Drug Development
Street1*:	2000 9th Ave. S
Street2:	
City*:	Birmingham
County:	
State*:	AL: Alabama
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	35205-3505

Phone Number*: (b) (3) (A), (b) (6) Fax Number:

E-Mail*: (b) (3) (A), (b) (6)

Credential, e.g., agency login: (b) (6)

Project Role*: Other (Specify) Other Project Role Category: Senior Project Manager

Degree Type: Ph.D. Degree Year: (b) (3)

Attach Biographical Sketch*: File Name: Biosketch_(b) (3) (A), (b)_v5_SR.pdf

Attach Current & Pending Support: File Name:

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 0636907050000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2019 **End Date*:** 02-28-2020 **Budget Period:** 1

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	Richard		Whitley		PD/PI		(b) (6)			24,796.00	7,488.00	32,284.00
2.	Dr.	Mark		Prichard		Co-PI					35,397.00	10,690.00	46,087.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **78,371.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Supervisor	(b) (6)			32,450.00	11,500.00	43,950.00
1	Research Technician				18,081.00	6,400.00	24,481.00
2	**Total Number Other Personnel**					**Total Other Personnel**	**68,431.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**146,802.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-17-042. Received Date: 2018-03-29T16:30:43.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 0636907050000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2019 **End Date*:** 02-28-2020 **Budget Period: 1**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**
Additional Equipment: File Name:	

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 0636907050000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2019 **End Date*:** 02-28-2020 **Budget Period: 1**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	38,300.00
2. Publication Costs	1,500.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	623,628.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Shipping and Courier Costs	250.00
9. Repair and Maintenance	2,000.00
10. Sequencing	4,500.00
Total Other Direct Costs	**670,178.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**819,980.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. MTDC (minus SR subaward)	48.5	196,352.00	95,231.00
2. 2. Subaward F&A (based on 1st $25,000 of total costs)	48.5	25,000.00	12,125.00
		Total Indirect Costs	**107,356.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number) DHHS, (b) (6)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**927,336.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	927,336.00

L. Budget Justification* File Name: Whitley_Project_4.1_Budget_justification_Final.pdf

(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Tracking Number: GRANT12598083 Funding Opportunity Number: RFA-AI-17-042. Received Date: 2018-03-29T16:30:43.000-04:00

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 0636907050000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2020 **End Date*:** 02-28-2021 **Budget Period: 2**

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1. Dr.	Richard		Whitley		PD/PI		(b) (6)			24,796.00	7,488.00	32,284.00
2. Dr.	Mark		Prichard		Co-PI					35,397.00	10,690.00	46,087.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **78,371.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Supervisor	(b) (6)			22,715.00	8,042.00	30,757.00
1	Research Technician				12,656.00	4,480.00	17,136.00
2	**Total Number Other Personnel**					**Total Other Personnel**	**47,893.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**126,264.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 0636907050000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2020 **End Date*:** 02-28-2021 **Budget Period: 2**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs		Funds Requested ($)*
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Tracking Number: GRANT12598083 Funding Opportunity Number: RFA-AI-17-042. Received Date: 2018-03-29T16:30:43.000-04:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 0636907050000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2020 **End Date*:** 02-28-2021 **Budget Period: 2**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	16,000.00
2. Publication Costs	2,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	807,669.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Shipping and Courier Costs	250.00
9. Repair and Maintenance	2,000.00
10. Sequencing	4,500.00
Total Other Direct Costs	**832,419.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**961,683.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. MTDC	48.5	154,014.00	74,697.00
		Total Indirect Costs	**74,697.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number) DHHS, (b) (6)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**1,036,380.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	1,036,380.00

L. Budget Justification* File Name: Whitley_Project_4.1_Budget_justification_Final.pdf

(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Tracking Number: GRANT12598083 Funding Opportunity Number: RFA-AI-17-042. Received Date: 2018-03-29T16:30:43.000-04:00

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 0636907050000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2021 **End Date*:** 02-28-2022 **Budget Period: 3**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	Richard		Whitley		PD/PI		(b) (6)			24,796.00	7,488.00	32,284.00
2.	Dr.	Mark		Prichard		Co-PI					35,397.00	10,690.00	46,087.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **78,371.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Supervisor	(b) (6)			22,715.00	8,042.00	30,757.00
1	Research Technician				12,656.00	4,480.00	17,136.00
2	**Total Number Other Personnel**					**Total Other Personnel**	**47,893.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**126,264.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 0636907050000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2021 **End Date*:** 02-28-2022 **Budget Period: 3**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 0636907050000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2021 **End Date*:** 02-28-2022 **Budget Period: 3**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	16,000.00
2. Publication Costs	2,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	807,669.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Shipping and Courier Costs	250.00
9. Repair and Maintenance	2,000.00
10. Sequencing	4,500.00
Total Other Direct Costs	**832,419.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**961,683.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. MTDC	48.5	154,014.00	74,697.00
		Total Indirect Costs	**74,697.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number) DHHS, (b) (6)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**1,036,380.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	1,036,380.00

L. Budget Justification* File Name: Whitley_Project_4.1_Budget_justification_Final.pdf

(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Tracking Number: GRANT12598083 Funding Opportunity Number: RFA-AI-17-042. Received Date: 2018-03-29T16:30:43.000-04:00

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 0636907050000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2022 **End Date*:** 02-28-2023 **Budget Period: 4**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	Richard		Whitley		PD/PI		(b) (6)			24,796.00	7,488.00	32,284.00
2.	Dr.	Mark		Prichard		Co-PI					35,397.00	10,690.00	46,087.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **78,371.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Supervisor	(b) (6)			22,715.00	8,042.00	30,757.00
1	Research Technician				12,656.00	4,480.00	17,136.00
2	**Total Number Other Personnel**					**Total Other Personnel**	**47,893.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**126,264.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-17-042. Received Date: 2018-03-29T16:30:43.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 0636907050000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2022 **End Date*:** 02-28-2023 **Budget Period: 4**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Tracking Number: GRANT12598083 Funding Opportunity Number: RFA-AI-17-042. Received Date: 2018-03-29T16:30:43.000-04:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 0636907050000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2022 **End Date*:** 02-28-2023 **Budget Period: 4**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	16,000.00
2. Publication Costs	2,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	819,881.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Shipping and Courier Costs	250.00
9. Repair and Maintenance	2,000.00
10. Sequencing	4,500.00
Total Other Direct Costs	**844,631.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**973,895.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. MTDC	48.5	154,014.00	74,697.00
		Total Indirect Costs	**74,697.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number) DHHS, (b) (6)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**1,048,592.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	1,048,592.00

L. Budget Justification* File Name: Whitley_Project_4.1_Budget_justification_Final.pdf

(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Tracking Number: GRANT12598083 Funding Opportunity Number: RFA-AI-17-042. Received Date: 2018-03-29T16:30:43.000-04:00

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 0636907050000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2023 **End Date*:** 02-29-2024 **Budget Period: 5**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	Richard		Whitley		PD/PI		(b) (6)			24,796.00	7,488.00	32,284.00
2.	Dr.	Mark		Prichard		Co-PI					35,397.00	10,690.00	46,087.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **78,371.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Supervisor	(b) (6)			22,715.00	8,042.00	30,757.00
1	Research Technician				12,656.00	4,480.00	17,136.00
2	**Total Number Other Personnel**					**Total Other Personnel**	**47,893.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**126,264.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 0636907050000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2023 **End Date*:** 02-29-2024 **Budget Period: 5**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Tracking Number: GRANT12598083 Funding Opportunity Number: RFA-AI-17-042. Received Date: 2018-03-29T16:30:43.000-04:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 0636907050000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2023 **End Date*:** 02-29-2024 **Budget Period: 5**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	16,000.00
2. Publication Costs	2,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	799,783.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Shipping and Courier Costs	250.00
9. Repair and Maintenance	2,000.00
10. Sequencing	4,500.00
Total Other Direct Costs	**824,533.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**953,797.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. MTDC	48.5	154,014.00	74,697.00
		Total Indirect Costs	**74,697.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number) DHHS, (b) (6)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**1,028,494.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	1,028,494.00

L. Budget Justification* File Name: Whitley_Project_4.1_Budget_justification_Final.pdf

(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Tracking Number: GRANT12598083 Funding Opportunity Number: RFA-AI-17-042. Received Date: 2018-03-29T16:30:43.000-04:00

Budget Justification – Project 4.1 UAB

Personnel

Richard J. Whitley, MD, PD/PI, (b) (6) calendar months (Years 1-5): Dr. Whitley will serve as the Project Lead for Project 4 investigating the inhibitors of influenza virus. Dr. Whitley is currently Distinguished Professor, Professor of Pediatrics, Microbiology, Medicine and Neurosurgery, and holds the Loeb Chair in Pediatrics in the School of Medicine at the University of Alabama at Birmingham (UAB). He has performed translational research for 40 years at UAB, and has directed multiple projects, including a currently funded CETR project to identify early leads for influenza therapies, and has investigated all aspects of antiviral drug development ranging from molecule selection, preclinical toxicology, ADME, animal models, resistance assessment, as well as human studies. Dr. Whitley is also the Director of the Alabama Drug Discovery Alliance (ADDA) – the collaboration between UAB and Southern Research focused on drug development for multiple diseases, and has worked closely with the Emory Institute for Drug Development (EIDD) to identify promising compounds for this proposed influenza project. He has worked with Dr. Prichard to develop the cap snatching assay for the proposed research, and will provide broad oversight of the project. He will serve as primary liaison with the Executive Committee, NIH and other external entities, including pharmaceutical companies to determine potential compounds to be developed. He will also work with international groups such as the WHO, CDC and IDSA to determine therapeutic needs and developments in the field of influenza research.

Mark Prichard, PhD. (b) (6) calendar months (Years 1-5): Dr. Prichard has conducted research in discovery and development of antiviral drugs for more than 25 years including 4 years of work on the live attenuated influenza virus vaccine at MedImmune Vaccines. For the past 12 years, he has served as the PI of an NIAID contract focused on the evaluation of compounds for antiviral activity against many DNA viruses. He also continues his work on influenza virus and published four reports on the virus in recent years. Dr. Prichard is the corresponding author or co-author of 116 publications, most of which are related to the in vitro evaluation of antivirals or on new targets for antiviral drug development. He also served as the Program Chair of the International Conference on Antiviral Research from 2013-2017. He currently serves on the Editorial Boards of Antiviral Research and the Journal of Infectious Disease and is an Editor for Antiviral Research. He will work closely with Southern Research and the Emory Institution for Drug Development (EIDD) on development of assays and evaluation of compounds, and will also look at sequencing and testing of drug resistance during the project.

(b) (3) (A), (b) (6) **MS, Laboratory Supervisor, (b) (6) calendar months- Year 1; (b) (6) calendar months Years 2-5):** (b) (3) (A), (b) (6) has (b) (3) years of experience working in a BSL-3 laboratory and has been part of the Antiviral testing program for the past 16 years. In addition to influenza, (b) (3) (A), (b) (6) has experience in laboratory work on a number of viruses including HIV, influenza virus, herpesviruses, poxviruses, papillomavirus, and adenovirus. (b) (3) (A), (b) (6) primarily works to determining in vitro drug efficacy using different endpoint methods (e.g., ELISA, CPE, plaque reduction, virus yield, hybridization, real time PCR) and under Biosafety levels 2 - 3. (b) (3) (A), (b) (6) has developed many of the assay systems currently used to determine antiviral activity of compounds, and has supervisory responsibilities over other personnel working in the BSL-3 facility. (b) (3) (A), (b) (6) will oversee the antiviral assays and day to day activities for this project in (b) (3) (A), (b) (6).

(b) (3) (A), (b) (6), **Research Technician, (b) (6) calendar months - Year 1; (b) (6) calendar months - Years 2-5):** (b) (3) (A), (b) (6) has been working in the laboratory since (b) (3) (A), (b) (6) with experience in many viruses including influenza virus, as well as DNA viruses including adenovirus, orthopoxvirus, and the herpesviruses. (b) (3) (A), (b) (6) developed and performs the influenza virus CPE reduction assays and also helped developed the virus yield reduction (VYR) assays for influenza virus. (b) (3) (A), (b) (6) is responsible for preparing all compound solutions, including reference compounds and notifying analysts when new compounds have arrived. (b) (3) (A), (b) (6) will provide general assistance with day to day operations for this project.

Supplies

Funds are requested in **Year 1** for General Lab materials and supplies including: tissue culture supplies (6,000); cells, reagents, probes and antibodies (29,300), Disposable labware and personal protection items PPE (2,000) and chemical, stains and solvents (1,000) for a total of $38,300. These supplies will be used to test antiviral activity of compounds to be provided by the EIDD. In **Years 2-5** as the antiviral activity and testing is completed, supplies will be reduced as follows: Tissue culture – 4,000; disposable labware and PPE – 2,000; chemicals, stains and solvents – 1,000; Cells and reagents – 9,000.

Travel

Funds (3,000 - Years 1-5) are requested to allow travel of the PD/PI and co-investigators to attend International Society of Antiviral Research conferences or other related scientific meetings to present results of the project, and collaborate with other project investigators.

Other Expenses

Funds are requested for shipping of materials ($250), publication costs ($1,500), and maintenance of project specific equipment ($2,000). Funding for sequencing core services ($4,500) is also included.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		391,855.00
Section B, Other Personnel		260,003.00
Total Number Other Personnel	10	
Total Salary, Wages and Fringe Benefits (A+B)		651,858.00
Section C, Equipment		0.00
Section D, Travel		15,000.00
1. Domestic	15,000.00	
2. Foreign	0.00	
Section E, Participant/Trainee Support Costs		0.00
1. Tuition/Fees/Health Insurance	0.00	
2. Stipends	0.00	
3. Travel	0.00	
4. Subsistence	0.00	
5. Other	0.00	
6. Number of Participants/Trainees	0	
Section F, Other Direct Costs		4,004,180.00
1. Materials and Supplies	102,300.00	
2. Publication Costs	9,500.00	
3. Consultant Services	0.00	
4. ADP/Computer Services	0.00	
5. Subawards/Consortium/Contractual Costs	3,858,630.00	
6. Equipment or Facility Rental/User Fees	0.00	
7. Alterations and Renovations	0.00	
8. Other 1	1,250.00	
9. Other 2	10,000.00	
10. Other 3	22,500.00	
Section G, Direct Costs (A thru F)		4,671,038.00
Section H, Indirect Costs		406,144.00
Section I, Total Direct and Indirect Costs (G + H)		5,077,182.00
Section J, Fee		0.00
Section K, Total Direct and Fee (I + J)		5,077,182.00

Tracking Number: GRANT12598083 Funding Opportunity Number: RFA-AI-17-042. Received Date: 2018-03-29T16:30:43.000-04:00

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 0069005260000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Southern Research

Start Date*: 03-01-2019 **End Date*:** 02-29-2020 **Budget Period:** 1

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.			(b) (3) (A), (b) (6)	Ph.D	Project Co-Investigator		(b) (6)			22,635.00	9,665.00	32,300.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **32,300.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	(b) (6)			47,947.00	20,473.00	68,420.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
2	Biologist				51,554.00	22,014.00	73,568.00
1	Suprv CompMgmt				3,249.00	1,387.00	4,636.00
1	Adv Bio IT Spec				3,100.00	1,323.00	4,423.00
5	**Total Number Other Personnel**					**Total Other Personnel**	**151,047.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**183,347.00**

RESEARCH & RELATED Budget (A-B) (Funds Requested)

Funding Opportunity Number: RFA-AI-17-042. Received Date: 2018-03-29T16:30:43.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 0069005260000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Southern Research

Start Date*: 03-01-2019 **End Date*:** 02-29-2020 **Budget Period: 1**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 1

ORGANIZATIONAL DUNS*: 0069005260000
Budget Type*: ❍ Project ● Subaward/Consortium
Enter name of Organization: Southern Research

Start Date*: 03-01-2019 **End Date*:** 02-29-2020 **Budget Period: 1**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	104,750.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**104,750.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**288,097.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. 1. OH- Salaries + Benefits	120.0	183,347.00	220,016.00
2. 2. G&A - Salaries + Benefits +OH+ODC	20.0	508,113.00	101,623.00
3. 3. FCCM -OH	7.3	183,347.00	13,384.00
4. 4. FCCM - G&A (less than 1% rate)	0.1	508,113.00	508.00
		Total Indirect Costs	**335,531.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number) DHHS/Darryl W. Mayes/301-492-4855

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**623,628.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	623,628.00

L. Budget Justification* File Name: Southern_Research_Project_4_Budget_Justification.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Tracking Number: GRANT12598083 Funding Opportunity Number: RFA-AI-17-042. Received Date: 2018-03-29T16:30:43.000-04:00

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 0069005260000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Southern Research

Start Date*: 03-01-2020 **End Date*:** 02-28-2021 **Budget Period: 2**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.			(b) (3) (A), (b) (6)	Ph.D	Project Co-Investigator		(b) (6)			22,635.00	9,665.00	32,300.00
2.					Ph.D	Project Manager					20,477.00	8,743.00	29,220.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **61,520.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	(b) (6)			47,947.00	20,473.00	68,420.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
2	Biologist				51,554.00	22,014.00	73,568.00
2	Scientist				4,144.00	1,769.00	5,913.00
4	Technical				10,845.00	4,629.00	15,474.00
2	Other support				8,868.00	3,787.00	12,655.00
11	**Total Number Other Personnel**					**Total Other Personnel**	**176,030.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**237,550.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 0069005260000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Southern Research

Start Date*: 03-01-2020 **End Date*:** 02-28-2021 **Budget Period: 2**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 2

ORGANIZATIONAL DUNS*: 0069005260000
Budget Type*: ❍ Project ● Subaward/Consortium
Enter name of Organization: Southern Research

Start Date*: 03-01-2020 **End Date*:** 02-28-2021 **Budget Period: 2**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	104,750.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Other charges	30,697.00
Total Other Direct Costs	**135,447.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**372,997.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. 1. OH- Salaries + Benefits	120.0	237,551.00	285,061.00
2. 2. G&A - Salaries + Benefits +OH+ODC	20.0	658,059.00	131,612.00
3. 3. FCCM -OH	7.3	237,551.00	17,341.00
4. 4. FCCM - G&A (less than 1% rate)	0.1	658,059.00	658.00
		Total Indirect Costs	**434,672.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS/Darryl W. Mayes/301-492-4855

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**807,669.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	807,669.00

L. Budget Justification* File Name: Southern_Research_Project_4_Budget_Justification.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Tracking Number: GRANT12598083 Funding Opportunity Number: RFA-AI-17-042. Received Date: 2018-03-29T16:30:43.000-04:00

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 0069005260000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Southern Research

Start Date*: 03-01-2021 **End Date*:** 02-28-2022 **Budget Period: 3**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	(b) (3) (A), (b) (6)			Ph.D	Project Co-Investigator		(b) (6)			22,635.00	9,665.00	32,300.00
2.					Ph.D	Project Manager					20,477.00	8,743.00	29,220.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **61,520.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	(b) (6)			47,947.00	20,473.00	68,420.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
2	Biologist				51,554.00	22,014.00	73,568.00
2	Scientist				4,144.00	1,769.00	5,913.00
4	Technical				10,845.00	4,629.00	15,474.00
2	Other support				8,868.00	3,787.00	12,655.00
11	**Total Number Other Personnel**					**Total Other Personnel**	**176,030.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**237,550.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-17-042. Received Date: 2018-03-29T16:30:43.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 0069005260000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Southern Research

Start Date*: 03-01-2021 **End Date*:** 02-28-2022 **Budget Period: 3**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 3

ORGANIZATIONAL DUNS*: 0069005260000
Budget Type*: ❍ Project ● Subaward/Consortium
Enter name of Organization: Southern Research

Start Date*: 03-01-2021 **End Date*:** 02-28-2022 **Budget Period: 3**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	104,750.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Other charges	30,697.00
Total Other Direct Costs	**135,447.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**372,997.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. 1. OH- Salaries + Benefits	120.0	237,551.00	285,061.00
2. 2. G&A - Salaries + Benefits +OH+ODC	20.0	658,059.00	131,612.00
3. 3. FCCM -OH	7.3	237,551.00	17,341.00
4. 4. FCCM - G&A (less than 1% rate)	0.1	658,059.00	658.00
		Total Indirect Costs	**434,672.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS/Darryl W. Mayes/301-492-4855

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**807,669.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	807,669.00

L. Budget Justification* File Name: Southern_Research_Project_4_Budget_Justification.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Tracking Number: GRANT12598083 Funding Opportunity Number: RFA-AI-17-042. Received Date: 2018-03-29T16:30:43.000-04:00

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 0069005260000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Southern Research

Start Date*: 03-01-2022 **End Date*:** 02-28-2023 **Budget Period: 4**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.			(b) (3) (A), (b) (6)	Ph.D	Project Co-Investigator		(b) (6)			22,635.00	9,665.00	32,300.00
2.					Ph.D	Project Manager					19,414.00	8,290.00	27,704.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **60,004.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	(b) (6)			47,947.00	20,473.00	68,420.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
2	Biologist				51,554.00	22,014.00	73,568.00
2	Scientist				3,893.00	1,663.00	5,556.00
4	Technical				12,713.00	5,428.00	18,141.00
2	Other support				8,280.00	3,535.00	11,815.00
11	**Total Number Other Personnel**					**Total Other Personnel**	**177,500.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**237,504.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 0069005260000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Southern Research

Start Date*: 03-01-2022 **End Date*:** 02-28-2023 **Budget Period: 4**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 4

ORGANIZATIONAL DUNS*: 0069005260000
Budget Type*: ❍ Project ● Subaward/Consortium
Enter name of Organization: Southern Research

Start Date*: 03-01-2022 **End Date*:** 02-28-2023 **Budget Period: 4**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	83,900.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Other charges	61,920.00
Total Other Direct Costs	**145,820.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**383,324.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. 1. OH- Salaries + Benefits	120.0	237,504.00	285,005.00
2. 2. G&A - Salaries + Benefits +OH+ODC	20.0	668,229.00	133,646.00
3. 3. FCCM -OH	7.3	237,504.00	17,338.00
4. 4. FCCM - G&A (less than 1% rate)	0.1	668,229.00	668.00
		Total Indirect Costs	**436,657.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS/Darryl W. Mayes/301-492-4855

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**819,981.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	819,981.00

L. Budget Justification* File Name: Southern_Research_Project_4_Budget_Justification.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Tracking Number: GRANT12598083 Funding Opportunity Number: RFA-AI-17-042. Received Date: 2018-03-29T16:30:43.000-04:00

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 0069005260000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Southern Research

Start Date*: 03-01-2023 **End Date*:** 02-29-2024 **Budget Period: 5**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	(b) (3) (A), (b) (6)			Ph.D	Project Co-Investigator		(b) (6)			22,635.00	9,665.00	32,300.00
2.					Ph.D	Project Manager					16,392.00	7,000.00	23,392.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **55,692.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	(b) (6)			47,947.00	20,473.00	68,420.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
2	Biologist				51,554.00	22,014.00	73,568.00
2	Scientist				3,140.00	1,341.00	4,481.00
4	Technical				10,690.00	4,564.00	15,254.00
2	Other support				6,881.00	2,938.00	9,819.00
11	**Total Number Other Personnel**					**Total Other Personnel**	**171,542.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**227,234.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-17-042. Received Date: 2018-03-29T16:30:43.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 0069005260000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Southern Research

Start Date*: 03-01-2023 **End Date*:** 02-29-2024 **Budget Period: 5**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 5

ORGANIZATIONAL DUNS*: 0069005260000
Budget Type*: ❍ Project ● Subaward/Consortium
Enter name of Organization: Southern Research

Start Date*: 03-01-2023 **End Date*:** 02-29-2024 **Budget Period: 5**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	94,275.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Other charges	57,929.00
Total Other Direct Costs	**152,204.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**379,438.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. 1. OH- Salaries + Benefits	120.0	227,234.00	272,681.00
2. 2. G&A - Salaries + Benefits +OH+ODC	20.0	652,119.00	130,424.00
3. 3. FCCM -OH	7.3	227,234.00	16,588.00
4. 4. FCCM - G&A (less than 1% rate)	0.1	652,119.00	652.00
		Total Indirect Costs	**420,345.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS/Darryl W. Mayes/301-492-4855

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**799,783.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	799,783.00

L. Budget Justification* File Name: Southern_Research_Project_4_Budget_Justification.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Tracking Number: GRANT12598083 Funding Opportunity Number: RFA-AI-17-042. Received Date: 2018-03-29T16:30:43.000-04:00

Key Personnel - Biochemistry and Molecular Biology

(b) (3) (A), (b) (6), **Ph.D.** *(Y1-Y5: (b) cal. months).* (b) (3) (A), (b) (6), Assistant Fellow in the Drug Discovery Division, Department of Infectious Diseases at Southern Research, will serve as Project Lead for Project 4. He has more than (b) years of experience in the field of bacteriology and phage biology at the National Center for Scientific Research (CNIC) in (b) (3) (A), (b) (6) and (b) additional years at SR working in the fields of virology and bacteriology. After he joined Southern Research, he has been involved in several projects not only in the field of bacteriology, but also in virology, specifically in the study of the mechanism of entry of Ebola virus and the designing and development of a new assay for identifying inhibitors of the VEEV protease. In this last field he is currently focused in the discovery of antiviral compounds against influenza less prone to develop viral resistance by targeting the RNA polymerase. (b) (3) (A), (b) (6) will supervise and perform available secondary confirmatory assays on hit compounds found in the primary (b) (3) (A), (b) (6) (b) (3) (A), (b) (6) In particular, *in vitro* assays to discern compound activity on polymerization, endonuclease activity, cap-binding or subunit interaction of the RdRp complex. He will assist in the designing of experiments to these aims and presentation of data and manuscripts, as well as participate in Project meetings.

Other Personnel- Biochemistry and Molecular Biology

(b) (3) (A), (b) (6), **Ph.D.** *(Y1-Y5: (b) cal. months).* (b) (3) (A), (b) is a Postdoctoral Researcher in (b) (3) (A), (b) (6) laboratory, who will conduct secondary screening of hit compounds against an influenza virus available *in vitro* polymerase assay (Aim 2). Additionally, (b) (3) (A), (b) will perform experiments to optimize and implement tertiary *in vitro* endonuclease and polymerase assays (Aim 2) to determine the mechanism of action of hit compounds on the RdRp complex (Aim 2).

(b) (3) (A), (b) (6), **M.S.** *(Y1: (b) cal. months; Y2-Y5: (b) cal. months).* (b) (3) (A), (b) (6) is a Biologist in the Drug Discovery Division. (b) will be responsible for growing and determining the titer of influenza virus stocks to make them available in downstream confirmatory assays and mode of action studies (Aim 1 & 2). (b) will also conduct secondary screening of hit compounds against different influenza virus subtypes including plaque reduction, cytopathic effect (CPE) hemaglutination, and neuramindase assays. (b) (3) (A), will also assist with BSL-3 evaluations of antiviral compounds for broad efficacy (Aim 2).

(b) (3) (A), (b) (6), **B.S.** *(Y1-Y5: (b) cal. months).* (b) (3) (A), is a Research Biologist in (b) (3) (A), (b) (6) laboratory who will assist in conducting secondary screening of hit compounds against an influenza virus (Aim 2). Additionally, (b) will perform experiments to optimize and implement tertiary cap-binding and subunit interaction assays (Aim 2) to determine the mechanism of action of hit compounds on the RdRp complex (Aim 2).

(b) (3) (A), (b) (6), **M.S.** *(Y1: (b) cal. months) Supervisor Compound Management.* (b) (3) (A), will be responsible for compound management and drugging for the biological assays.

(b) (3) (A), (b) (6), **B.S.** *(Y1: (b) cal. months).* (b) (3) (A), (b) (6), Advanced Biologist Informatics Specialist, will be responsible for importing the data from the plate readers into the analysis software and generating data reports.

Key Personnel – Animal MTD Studies

(b) (3) (A), (b) (6) ***Ph.D.*** *(Y2-Y3:* (b) (6) *cal. months; Y4:* (b) (6) *cal. months; Y5:* (b) (6) *cal. months).* (b) (3) (A), (b) (6) is a multi-disciplinary research scientist with over (b) (3) years of experience in bacteriology, virology, immunology and molecular biology, and is experienced in designing plans for validating or qualifying bioanalytical and immunological assays for analyzing non-clinical and clinical samples. (b) (3) (A), (b) (6) (b) (3) (A), (b) (6) designs and executes validations or qualifications and manages sample analysis in partnership with both commercial and government clients. Prior to joining SR in 2015, (b) (3) (A), (b) (6) In addition, he supervised and performed a broad range of procedures using novel and standard techniques and methods including refining a number of viral and bacterial animal models. Viral animal models were primarily mouse and ferret influenza models using seasonal, pandemic and highly pathogenic avian influenza strains. He will be responsible for the overall conduct of a multidisciplinary team effort to complete the proposed MTD studies.

Other Personnel– Animal MTD Studies

(b) (3) (A), (b) (6), *(Y2-Y3:* (b) (6) *cal. months; Y4:* (b) (6) *cal. months; Y5:* (b) (6) *cal. months).* (b) (3) (A) (b) Associate Project Leader, will assist (b) (3) (A), (b) (6) in program management function, including budgets, resource management, and reporting to ensure that the program's deliverables are provided on time and on budget.

In Vivo technical personnel: SR's fully-qualified Operations team, a staff of animal care and equipment technicians, will attend to all study requirements associated with the MTD studies, providing routine animal husbandry and support.

- (b) (3) (A), (b) (6) *(Y2-Y3:* (b) (6) *cal. months; Y4:* (b) (6) *cal. months; Y5:* (b) (6) *cal. months), In Vivo* Supervisor
- (b) (3) (A), (b) (6) *(Y2-Y3:* (b) (6) *cal. months; Y4:* (b) (6) *cal. months; Y5:* (b) (6) *cal. months), In Vivo* Technician
- (b) (3) (A), (b) (6) *(Y2-Y3:* (b) (6) *cal. months; Y4:* (b) (6) *cal. months; Y5:* (b) (6) *),* Veterinary Technician
- (b) (3) (A), (b) (6) *(Y2-Y3:* (b) (6) *cal. months; Y4:* (b) (6) *cal. months; Y5:* (b) (6) *cal. months),* Dose Formulation
- (b) (3) (A), (b) (6) *(Y2-Y3:* (b) (6) *cal. months; Y4:* (b) (6) *cal. months; Y5:* (b) (6) *cal. months),* Prosector
- (b) (3) (A), (b) (6) *(Y2-Y3:* (b) (6) *; Y4:* (b) (6) *cal. months; Y5:* (b) (6) *cal. months),* Sample Prep

(b) (3) (A), (b) (6) *(Y2-Y3:* (b) (6) *cal. months; Y4:* (b) (6) *cal. months; Y5:* (b) (6) *cal. months).* (b) (3) (A) (b) (6) Report Writer, will be responsible for report writing and quality control. (b) (3) will be responsible for compiling the QC'd data into formatted draft reports to be completed by the Project Manager/Study Director. Report preparation and oversight will be conducted under the supervision of our Director of Report Writing, Quality Control and Validation. QC Inspectors ensure the integrity and quality of data by providing 100% reviews at critical points throughout a study.

The teams of professionals at Southern Research as outlined above are identified as a competent and experienced pool of individuals. We have identified these individuals due to

their capabilities, but understand staff changes do occur. Given the timeframe of this Task Order, Southern Research commits to resources of these same capabilities or greater should staffing changes occur.

Other (Non-Labor) Direct Costs *(Y1: $104,750; Y2-Y3: $135,447, annually; Y4: $145,720; Y5: $152,204)*

Materials and Supplies *(Y1: $104,750; Y2-Y3: $104,750, annually; Y4: $83,800; Y5: $94,275).* These include labor and materials for cell culture, biochemical assay performance, and general supply costs. These estimates are based upon catalog pricing from commercial vendors (e.g., (b) (3) (A), (b) (6) etc.), historical patterns of laboratory usage, and consumables we already have available from Southern Research. Consumables will include chemicals like buffers, acids and bases, and molecular reagents such as antibodies and flow cytometry reagents. Disposables will include flasks, pipette tips, tissue culture plates, and other miscellanea consumables which are fundamental to the proposed studies.

Animal Costs/MTD Studies *(Y2-Y3: $30,697, annually; Y4: $47,401, Y5: $43,410).* These costs cover animals, animal care per diems, dosing supplies, bioanalytical supplies, necropsy materials, and all other supporting materials required for MTD Animal Studies.

BSL-3 *(Y4: $14,518; Y5: $14,518).* BSL-3 laboratory charges are included for 36 days of operations and incubations.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		271,036.00
Section B, Other Personnel		852,149.00
Total Number Other Personnel	49	
Total Salary, Wages and Fringe Benefits (A+B)		1,123,185.00
Section C, Equipment		0.00
Section D, Travel		0.00
1. Domestic	0.00	
2. Foreign	0.00	
Section E, Participant/Trainee Support Costs		0.00
1. Tuition/Fees/Health Insurance	0.00	
2. Stipends	0.00	
3. Travel	0.00	
4. Subsistence	0.00	
5. Other	0.00	
6. Number of Participants/Trainees	0	
Section F, Other Direct Costs		673,668.00
1. Materials and Supplies	492,425.00	
2. Publication Costs	0.00	
3. Consultant Services	0.00	
4. ADP/Computer Services	0.00	
5. Subawards/Consortium/Contractual Costs	0.00	
6. Equipment or Facility Rental/User Fees	0.00	
7. Alterations and Renovations	0.00	
8. Other 1	181,243.00	
9. Other 2	0.00	
10. Other 3	0.00	
Section G, Direct Costs (A thru F)		1,796,853.00
Section H, Indirect Costs		2,061,877.00
Section I, Total Direct and Indirect Costs (G + H)		3,858,730.00
Section J, Fee		0.00
Section K, Total Direct and Fee (I + J)		3,858,730.00

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

Expiration Date: 03/31/2020

1. Vertebrate Animals Section

Are vertebrate animals euthanized? ● Yes ○ No

If "Yes" to euthanasia

Is the method consistent with American Veterinary Medical Association (AVMA) guidelines?

● Yes ○ No

If "No" to AVMA guidelines, describe method and provide scientific justification

2. *Program Income Section

*Is program income anticipated during the periods for which the grant support is requested?

○ Yes ● No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period *Anticipated Amount ($) *Source(s)

Tracking Number: GRANT12598083 Funding Opportunity Number: RFA-AI-17-042 . Received Date: 2018-03-29T16:30:43.000-04:00

PHS 398 Cover Page Supplement

3. Human Embryonic Stem Cells Section

*Does the proposed project involve human embryonic stem cells? ○ Yes ● No

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, check the box indicating that one from the registry will be used:

❑ Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Cell Line(s) (Example: 0004):

4. Inventions and Patents Section (Renewal applications)

*Inventions and Patents: ○ Yes ○ No

If the answer is "Yes" then please answer the following:

*Previously Reported: ○ Yes ○ No

5. Change of Investigator/Change of Institution Section

❑ Change of Project Director/Principal Investigator

Name of former Project Director/Principal Investigator

Prefix:

*First Name:

Middle Name:

*Last Name:

Suffix:

❑ Change of Grantee Institution

*Name of former institution:

Tracking Number: GRANT12598083 Funding Opportunity Number: RFA-AI-17-042 . Received Date: 2018-03-29T16:30:43.000-04:00

PHS 398 Research Plan

OMB Number: 0925-0001
Expiration Date: 03/31/2020

Introduction	
1. Introduction to Application (for Resubmission and Revision applications)	
Research Plan Section	
2. Specific Aims	Project_4_final_Aims.pdf
3. Research Strategy*	Project_4_final_Strategy.pdf
4. Progress Report Publication List	
Other Research Plan Section	
5. Vertebrate Animals	Vertebrate_Animals_Influenza.pdf
6. Select Agent Research	Project_4_Select_Agents_final.pdf
7. Multiple PD/PI Leadership Plan	
8. Consortium/Contractual Arrangements	
9. Letters of Support	
10. Resource Sharing Plan(s)	Resource_sharing_plan_influenza.pdf
11. Authentication of Key Biological and/or Chemical Resources	Authentication_of_Biological_Reagents_Influenza.pdf
Appendix	
12. Appendix	

Tracking Number: GRANT12598083 Funding Opportunity Number: RFA-AI-17-042. Received Date: 2018-03-29T16:30:43.000-04:00

SPECIFIC AIMS

Specific aims: The overall goal of this project is to identify new therapies that target influenza virus replication. The global health burden of annual influenza infections and periodic epidemics coupled with the emergence of avian influenza viruses (AIVs), like H5NX and H7N9, highlight the urgent need for new effective treatments. A primary concern with the current influenza therapies (M2 and neuraminidase inhibitors [NAIs]) is the development of resistance mutations that can negate therapeutic benefit. Both published evidence and clinical experience suggest strongly that targeting the influenza virus RNA dependent RNA polymerase (**RdRp**) complex is a rational approach for antiviral therapy. This complex is responsible for many viral functions, including 5´ cap recognition, endonucleolytic cleavage, RNA synthesis, and polyadenylation. Clearly, the trimeric RdRp complex provides multiple functional domains as targets for antiviral drug therapy and combination therapy. Agents can target the distinct functions and, theoretically, reduce the minimize development of resistance since resistance mutations would likely reduce the fidelity of the RdRp. One oral agent targeting the endonuclease domain, baloxavir, has shown efficacy in uncomplicated influenza and was recently approved in Japan for treating influenza in adults and children. A second oral agent targeting the 5´ cap binding domain, pimodivir, has shown antiviral activity in uncomplicated influenza and is advancing in clinical development. A third oral agent, favipiravir, targeting the polymerase activity has been approved in Japan for treating novel influenza strains not inhibited by NAIs.

We have recently identified several potent molecules from the Emory Institute for Drug Discovery (**EIDD**), Gilead Sciences (**GS**), and Southern Research (**SR**). This application and research team provides the medicinal chemistry expertise, follow up assays, and *In vivo* experience to transform active hits into lead compounds and advance them rapidly into animal models of influenza virus infection. The public private partnership with EIDD and GS leverages the proposed developmental program and promises to yield new classes of highly active molecules that target the RdRp complex. **We propose the following Specific Aims:**

Aim 1. Identify potent influenza compounds targeting RdRp complex functions

A. Continue hit to lead optimization of the active compounds from EIDD, GS, and SR.

Hypothesis and rationale: We hypothesize that hits identified from ongoing studies that target the RdRp complex can serve as the basis for a lead optimization program. This includes novel chemical classes from EIDD, SR, and GS. We will use an iterative medicinal chemistry effort that addresses potency (antiviral activity) as part of the Medicinal Chemistry and Lead Development Core (**MCLDC**). The focus will be on the essential activities of this complex, which provides fertile ground for drug discovery due to the number of conserved functional domains, and interactions that could be targeted. Our goal is to identify compounds directed against the conserved functions of the RdRp that are active against multiple subtypes of influenza virus.

Approach: The initial hits from EIDD, GS, and the SR compound collection will undergo hit to lead chemistry. Additional analogs will be evaluated and related compounds synthesized. Compounds with effective concentrations below 5 μM will be evaluated further to assess their activity against multiple subtypes of the virus.

Aim 2: Define the spectrum of antiviral activity and define mechanism of action of the lead compounds

A. Define spectrum of antiviral activity against wild-type, AIV, and NAI resistant strains of influenza virus
B. Characterize the mechanism of action (MOA) against RdRp functions
C. Map molecular determinants of resistance and their impact on susceptibility and replication fitness
D. Perform combination studies and evaluate lead molecules from other three Projects

Hypothesis and rationale: Active molecules presented here will be evaluated to confirm their activity against multiple subtypes of the virus including AIVs and preliminary data already have identified compounds that appear to inhibit at least three distinct RdRp functions. Additional assays in an appropriate setting of primary human small airway epithelial cells and biochemical assays will identify domains targeted by the most active compounds.

Experimental strategy: Several secondary and tertiary assays are in place to identify rapidly compounds that specifically inhibit the functions of the RdRp complex. The lead compounds also will be used to generate resistant isolates so that the molecular determinants of resistance can be mapped and confirmed in engineered viruses.

Aim 3: *In vivo* evaluation and bioavailability studies.

A. Perform maximum tolerated dose and pharmacokinetic studies in mice
B. Evaluate efficacy against mouse-adapted A/California/04/2009 (H1N1) infection in mice
C. Characterize efficacy of lead compound in ferrets infected with A/California/04/2009 (H1N1)

Hypothesis and rationale: Pharmacokinetic, bioavailability, distribution, and clearance studies will prioritize the best chemical scaffolds for further development and help inform the design of studies in mice and ferrets for pivotal efficacy experiments *In vivo*.

Experimental strategy: Compounds with the best efficacy from Aims 1 and 2 will be prioritized to identify molecules with drug-like properties for efficacy studies in murine and ferret models of influenza virus infection.

SIGNIFICANCE AND IMPACT

We have identified three new classes of influenza virus inhibitors and active molecules that target three distinct functions of the RdRp complex, providing completely novel opportunities for therapeutic control of contemporary as well pandemic influenza viruses. These inhibitors appear to target RNA replication fidelity, endonuclease activity, and cap snatching of the RdRp complex. We will optimize these and other novel molecules in collaboration with outstanding corporate partners at GS and EIDD, and will establish efficacy in animal models of influenza virus infection. The premise of these studies is that potent new molecules that we identified in human cells including A549 and primary small airway epithelial cells are active an appropriate metabolic and biological context. Most previous studies have utilized canine MDCK cells that metabolize nucleosides differently than human cells. This will help ensure that their efficacy will translate into infections of the human host. New molecules identified will add to the therapeutics available for the treatment of influenza virus infections and will be particularly valuable as they appear to be novel structures that inhibit different molecular targets in the virus.

Influenza remains an ongoing public health problem because of morbidity and mortality associated with seasonal epidemics as well as the threat of a global pandemic. Since October 1, 2017, there have been 21,279 laboratory confirmed influenza associated hospitalizations in the United States reported to the CDC. The highest hospitalization rate was among individuals sixty-five years and older (322.7 per 100,000) (1). These rates are higher than end of season rate for the 2014 – 2015 season, and these levels likely underestimate the total hospitalization rate across the country. Notably, a total of 114 influenza associated pediatric deaths have occurred for the week ending February 3, 2018 (2).

Influenza virus pandemic potential and the need for new therapies. Orthomyxoviruses, such as influenza virus, have negative-sense, segmented RNA genomes. The emergence of influenza A viruses from the aquatic avian reservoir occurs intermittently to cause zoonotic infections and contribute genes to novel influenza A viruses causing unpredictable pandemics (reviewed in (1, 3)). The transmissibility of the virus and the susceptibility of the population are among the factors that determine the extent to which new strains circulate in the general population. Three common influenza A virus subtypes (H1, H2, and H3) predominate in humans, although occasionally other subtypes are acquired as zoonoses and include subtypes such as H5 and H7. Similarly, two major subtypes of neuraminidases (N1 and N2) commonly circulate in the population with additional subtypes occasionally introduced in a context of zoonotic infections with novel HA subtypes. The genetic diversity is high among influenza A viruses and can result in periodic changes in the antigenic characteristics of the HA and NA as well as more extensive shifts of their genetic segments. Antigenic drifts are associated with seasonal epidemics (e.g., the dominant H3N2 strain during the 2017-18 season has the 1968 pandemic virus as its ancestor) and localized outbreaks of varying extent, whereas more divergent antigenic shifts are associated with pandemics. Transmission of highly pathogenic AIV strains to humans occurs occasionally. Recent laboratory confirmed zoonotic infections with H5N1, H5N6, and H7N9 strains are associated with startlingly high levels of morbidity and mortality, and thus, are a significant public health concern (4, 5). Of these, H7N9 strains rank as the most likely strain to cause a pandemic (6, 7). Antiviral therapy of influenza A strains can give rise to resistant infections that are efficiently transmitted and, in some instances, can become the predominant phenotype among circulating strains of some viruses. Almost all currently circulating influenza A viruses are resistant to adamantanes, and in 2007-8 oseltamivir-resistant seasonal H1N1 virus (due to H275Y substitution in NA) circulated globally. This underscores the need for the development of new antiviral drugs for the treatment of influenza viruses as well as a therapeutic strategy to reduce the emergence of resistant strains (reviewed in (8)).

Advances in influenza antiviral therapy. Since the emergence of AIVs as causes of relatively frequent zoonotic infections, concerns exist that a pandemic is imminent. Two classes of compounds have been licensed in the U.S. for the treatment of influenza, namely: (1) M2 ion channel inhibitors, adamantane derivatives (amantadine and rimantadine) and (2) neuraminidase inhibitors (oseltamivir, zanamivir, and peramivir). A long-acting inhaled neuraminidase inhibitor, laninamivir, recently was approved in 2010 in Japan but remains investigational elsewhere. The M2 inhibitors are only effective against A strains of influenza virus; however, by 2005 in the United States, 92% of influenza A (H3N2) isolates had developed high level resistance to this class of drugs. Currently, >99% of both H3N2 and H1N1 strains exhibit high level resistance to these drugs. The Centers for Disease Control and Prevention (CDC) recommends that neither amantadine nor rimantadine be used for the treatment or chemoprophylaxis of influenza in the US. The NAIs represent the only class of approved therapies that are active against currently circulating strains of the virus globally. Oral oseltamivir, inhaled zanamivir, and intravenous peramivir are currently effective neuraminidase inhibitors; yet the frequency of resistance may be increasing among currently circulating strains of influenza virus (8). Frequencies of resistance

to oseltamivir in A(H1N1), H3N2 strains, and B strains were 1.8%, 0.5%, and 0.6%, respectively, among adults and somewhat higher on children (~3%) during the 2015-2016 season. While experience with H5N1 strains is limited, resistance to oseltamivir can occur during the treatment (9), and the presence of variants within quasispecies that exhibit reduced susceptibility to oseltamivir in AVI strains of waterfowl is concerning (10). Additional drugs are in late stage clinical trials including nitazoxanide and DAS181 that inhibit cellular targets (11), but these have shown modest antiviral efficacy in controlled studies.

Three compounds targeting three distinct RdRp functions are in advanced stages of development in the U.S. These include the pyrazinecarboxamide RdRp polymerase inhibitor, favipiravir (T-705), the PB2 cap binding inhibitor pimodivir (JNJ63623872, VX-787), and the PA endonuclease inhibitor, baloxavir (S-033188) (reviewed in (11, 12)). Favipiravir was approved in Japan in 2014, but is not used clinically; two phase 3 treatment studies in adults with uncomplicated influenza have been completed in the U.S., but the efficacy results and safety concerns were inconsistent and unlikely to lead to licensure (13). The endonuclease inhibitor, baloxavir, was recently approved in Japan and was shown to reduce viral titers and levels of RNA significantly faster than oseltamivir, but the time to the cessation of all symptoms was not superior (14). Importantly, early clinical results suggest that variants with reduced susceptibility to this compound may emerge rapidly, namely in up to ~10% of H3N2-infected adults. The cap binding inhibitor, pimodivir, has completed phase 2 studies in both outpatients and hospitalized patients with evidence for antiviral efficacy. Currently, it is in Phase 3 clinical trials testing its efficacy when added to standard of care (NAI in most centers) (15-17). Together, these drugs highlight not only the multiple functions of the RdRp complex and the critical nature of these functions in viral replication, but also their ability to be targeted with effective antiviral therapies. To date, none of these compounds has been approved by the U.S. FDA, but they highlight the feasibility of targeting the RdRp complex.

INTRODUCTION TO SPECIFIC COMPOUNDS

There are multiple functional domains in the trimeric influenza RdRp complex (PB1, PB2, and PA) with significantly conserved regions and structural motifs among strains (18, 19). For example, the endonuclease function of PA is in the N-terminus of this protein, and 30 amino acids in this domain are highly conserved in influenza A, B, and C strains, including the four that are required for metal binding and the catalytic lysine (20). There are also 27 conserved residues in the cap-binding domain of PB2, and two are essential for binding of the m7GpppN cap. Furthermore, four conserved motifs are present in all strains of influenza virus PB1 catalytic core whereby mutagenesis of key amino acids abrogates polymerase activity (21, 22). Clearly, the highly conserved functional domains provide excellent targets for the development of new therapeutics for this virus.

The replication of influenza is a complex process that presents many opportunities for the development of novel antiviral therapies (23). The active molecules in this application were derived from novel and diverse compound collections and identified by assays in human cells+ designed to enrich hits with inhibitors that target RdRp complex functions. Following infection, the viral ribonucleoproteins (vRNPs) are transported to the host cell nucleus. The heterotrimeric RdRp complex together with the nucleoprotein (NP) initiate the replication process (18, 24). The synthesis of viral RNA does not require a primer, but rather, the viral RdRp initiates RNA synthesis internally on viral RNA. The RdRp also can direct mRNA synthesis through a process called cap snatching (reviewed in (18)). This process begins when the RdRp is in a pre-initiation state with the PB2 cap-binding and PA endonuclease domains in alignment so that a host capped RNA can be bound and cleaved ~13 nucleotides from the cap site. The cap binding domain then rotates to present the newly generated 3′ terminus to the polymerase active site, and polyadenylation occurs by a stuttering mechanism. The multiple conformations of the heterotrimeric RdRp helps direct its many different functions including 5′ cap-recognition, endonuclease activity, transcription and polyadenylation of viral mRNAs, protein-protein interactions, and synthesis of positive strand cRNA and negative strand genomic vRNA. Novel active compounds presented herein inhibit at least three of these essential processes.

(b) (4)

Hit compounds from collaborations with EIDD and GS. Ongoing studies with EIDD and GS evaluated small numbers of selected molecules with a high probability of inhibiting the replication of influenza virus. EIDD has assembled a library of approximately 1,000 nucleoside and nucleotide analogs, and from these, a subset of 25 coded compounds was evaluated in a series of CPE-based assays in MDCK cells infected with H1N1, H3N2, and B strains of influenza virus. One of the compounds, EIDD 1931, inhibited the replication of the H1N1 strain with a potency (EC_{50}) of less than 1 μM (**Fig. 1**) and also inhibited an H3N2 and B strain with EC_{50} values of 3.5 and 0.36 μM, respectively. Activity was also observed against A/VN/1203/04 H5N1. Based on work in **Projects 1-3,** this compound has broad-spectrum antiviral activity against many viruses,

including alphaviruses (Venezuelan equine encephalitis (VEEV) and chikungunya viruses), coronaviruses, and hepatitis C virus (25). The mechanism of action of EIDD 1931 against VEEV is mediated through the viral nsP4 protein (see **Project 2**), a component of the replication complex, and leads to the accumulation of mutations in viral RNA. This compound is predicted to inhibit influenza virus in a similar manner and by a mechanism similar to that reported for favipiravir (26, 27). A total of six additional compounds exhibited EC_{50} values of <10 μM against at least one influenza virus. Similarly, a set of 12 coded compounds was obtained from GS and evaluated against a nanoluc reporter virus (A/California/04/2009(H1N1) PASTN) (28) in primary human small airway epithelial cells. This approach identified four nucleosides and two nonnucleosides with EC_{50} values of <10 μM. Interestingly, four of the active nonnucleosides were structurally similar to GS 490260 (**Fig. 1**), which was reported previously to inhibit PA endonuclease activity by chelating metal ions in the amino terminus of the enzyme (29).

We recently developed an influenza virus **cap snatching assay** to detect inhibitors of this central function of the RdRp complex. Recent work suggested that the RdRp complex exhibits a preference for capped small noncoding host RNAs and identified preferred substrates including the spliceosome small nuclear RNA U1 and U2 (30). Since these were shown to represent approximately 1% of snatched host mRNA fragments, it was possible to develop a qPCR assay to quantify these specific products of the cap snatching process in infected cells. This assay quantifies levels of a specific chimeric U2-PB2 mRNA that represents approximately 1% of all PB2 RNAs in infected A549 cells at 24 h following infection. (b) (4)

We used this assay to evaluate 25 EIDD nucleosides and results are presented in **Table 1** together with antiviral activity in a CPE based assay (all exhibited CC_{50} values >30 μM). The control compound, pimodivir (VX-787), efficiently inhibited viral replication as well as the production of mRNA and as was expected given its inhibition of cap binding by PB2 (16). Another control compound, favipiravir (T-705), potently inhibited virus replication but did not exhibit an appreciable effect on levels of the influenza virus mRNAs. These results are exciting as T-705 is incorporated into viral RNA and inhibits RNA polymerase activity to some degree, but largely inhibits virus replication through "mutational meltdown" (27, 31), thus the assay can clearly distinguish between inhibition of polymerase activity and specific inhibitors of the cap snatching process. Both ribavirin and oseltamivir controls inhibited virus replication, but were inactive in the cap snatching assay (data not shown). Data from EIDD 1931 were similar to those of T-705, with EC_{50} values 100-fold higher in the cap snatching assay than the antiviral assay and are consistent with its reported effects on replication fidelity in VEEV. More importantly, three compounds with measurable antiviral activity also inhibited the production of influenza mRNA in the cap snatching assay with an efficacy within two-fold of their antiviral activity, suggesting that they inhibited early steps in the cap snatching process. Interestingly, the active molecules were phosphonate ester nucleotides and a guanine analog raising the intriguing possibility that they might be inhibiting the 7-methyl-GTP binding function of PB2.

These data taken together show that active molecules in **Fig. 1** and **Table 1** inhibit RdRp complex functions by up to **three different mechanisms;** mutational meltdown (**EIDD-1931**), endonuclease inhibition (**GS490-260**), and cap snatching (**EIDD-02838**). These molecules are exciting candidates for further development and some, such as EIDD-1931 and its prodrug, can be advanced into spectrum of activity studies and animal models by Year 2 (**Aims 2, 3**). Other active molecules in **Figure 1** and **Table 1, 2** are excellent candidates for advancement into the medicinal chemistry program in **Aim 1**. Additional novel molecules (data not shown), also identified in the previous CETR, were identified in human cells and will also be advanced as candidates in the medicinal chemistry program.

Hit compounds from the SR HTS campaign. Active compounds were identified from the SR compound collection. Prior to 2013, SR performed a HTS campaign of 250,500 compounds against three strains of influenza A virus (H1N1, H3N2 and H5N1) using CPE inhibition based assays, which provided an initial set of compounds

(b) (4)

for the **MCLDC** to analyze and select analcgs for further studies. The hit compounds were analyzed for structural liabilities using the Pan Assay Interference Compounds (PAINS) filter, a computational approach to identify false positives or hits that may represent unfavorable chemotypes (32). For instance, some chemotypes are known to promiscuously interact with host proteins, thus hindering its subsequent mechanism of action and target identification efforts (33). After filtering out promiscuously active and undesirable compounds, the remaining hits were analyzed for structural similarities within the set using clustering analysis. From this analysis, structural clusters were identified that represented chemical diversity and novel chemotypes representing cluster centroid and cluster members. All chemotypes were assembled and subjected to substructure searching based on these criteria using scripts that automate similarity queries and associated quality scores were generated. These hits were then ranked according to antiviral activity, cytotoxicity, selectivity index (SI = CC_{50}/EC_{50}) and structural desirability including Lipinski's Rules. A set of 40 compounds was selected and fresh material was acquired or synthesized for reconfirmation in antiviral and cell cytotoxicity assays. Structural integrity (^{1}H NMR, LCMS) and purity (HPLC) of these hits were determined before submitting to the HTS core for assessment. Two hits **SRI-34993** [EC_{50} (H1N1) = 3.2 μM, EC_{50} (H3N2) = 2.9 μM, CC_{50} > 70 μM, MLM = 2.3 min] and **SRI-33941** [EC_{50} (H1N1) = 28 μM, EC_{50} (H3N2) = 19 μM, EC_{50} (H5N1) = 28 μM, CC_{50} > 70 μM, MLM = 3.2 min] were identified and selected for follow-up chemistry to improve the potency and *in vitro* adsorption, distribution, metabolism and excretion (ADME) properties. A total of 33 analogs of SRI-34993 and SRI-33941 were evaluated for efficacy, but very little improvement in potency was observed and this series was discontinued.

Another HTS campaign was performed on 196,721 unique compounds against H1N1 and H3N2 strains of Influenza A virus using the enzyme-linked virus inhibitor reporter assay (ELVIRA)®. This provided 892 hits after PAINS filtering, which were then tested in a newly developed immunofluorescence (IF) assay measuring surface M2 protein levels using an H3N2 strain infected A549 cells. A total of 25 hits were selected using the criteria EC_{50} < 15 μM and CC_{50} > 50 μM, reproduced and analyzed for purity (HPLC) and integrity (HR-MS and ^{1}H NMR) before reconfirmation in the IF assay. Two compounds (SRI-19656 and SRI-36768) (**Table 2**) had activity reconfirmed in this assay with viral titer reduction (VTR) > 4 logs at 10 μM. These compounds, together with those outlined in **Fig. 1 and Table** 1 are ready for advancement into the **MCLDC**.

Combination treatment to enhance efficacy. While highly potent therapies, such as baloxavir, rapidly reduce viral load, they failed to accelerate symptom resolution of infected individuals with uncomplicated influenza (14). However, uncomplicated influenza in adults is associated with short duration of viral replication, such that upper respiratory tract viral loads are already decreasing at the time of presentation for care. Such observations suggest that pro-inflammatory host responses are contributing to illness. In contrast, more protracted viral replication, especially in the lower respiratory tract, is a common feature of serious influenza among hospitalized and critically ill patients, including those with A(H1N1)pdm09 illness or zoonotic AIV infections, and in high-risk populations like infants/young children and immunocompromised hosts. In such circumstances, combination therapy will need to be considered with at least two inhibitors (34) and with other modalities affecting deleterious host responses (35-37). Combination therapy is also predicted to help reduce the onset of resistant infections.

INNOVATION

The discovery of molecules that inhibit influenza virus replication by novel mechanisms is essential to complement existing antiviral therapies, which are continually becoming less effective due to the propagation of resistance mutations. This collaborative public private partnership, that includes EIDD, GS, SR, and UAB under the CETR umbrella of AD3C, will enhance the discovery of novel inhibitors by the development of unique molecules that have 'drug-like' properties. In addition to selecting molecules with potency and chemical tractability, we will also focus primarily on compounds that target functions of the RdRp. We have developed assays that assess distinct facets of the influenza RdRp complex and these were used to identify compounds that target the influenza polymerase activities. While other investigators have targeted polymerase activity, a novelty of this application lies in the development of unique molecules identified through the evaluation of proprietary compound collections for molecules that inhibit the replication of influenza virus in human cells and inhibit at least one of the many functions of the RdRp complex.

New molecules from new assays. The active compounds described above were identified through both HTS and a set of novel assays that benefited from recent technological advances and an improved understanding of RdRp complex functions in human cells. This included a luciferase reporter strain of influenza A virus A/California/10/2009(H1N1) PASTN (13) that was used in a primary human small airway epithelial cell assay against a selected set of molecules from the GS collection. This approach identified compounds and analogs that inhibit PA endonuclease activity. Another qPCR assay was used to evaluate 26 compounds from EIDD in a qPCR cap snatching process in infected A549 cells, and comparing the data to a counter screen in standard CPE inhibition assays in MDCK cells. Finally, a validated HTS primary assay utilizing an A549 reporter cell line expressing RdRp-dependent luciferase activity was used to screen 196,721 small molecules from novel libraries and identified 892 confirmed hits. Together these assays identified potent molecules that appear to inhibit at least three functions of the RdRp. The approach resulted in the discovery of novel compounds that target the RdRp and are poised for development into new therapies for influenza virus infections.

Cooperative Development path. With active molecules in hand, investigators at UAB, SR, EIDD, and GS are poised to advance promising molecules into the **MCLDC** outlined herein (**Fig. 2**). Continuous evaluation of progress and timelines is planned, and realistic **Go/No-Go** decisions will be made as the described medicinal chemistry strategies are executed and in conjunction with our advisory committees. Active molecules described here appear to target essential functions of the RdRp and promise to enrich the mechanistic diversity of the pool of compounds selected for further development. Most importantly, lessons learned from the development of active molecules from all of the projects will help to improve the efficiency of medicinal chemistry efforts and *In vivo* efficacy studies.

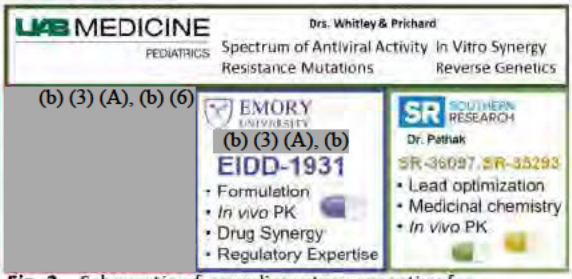


Fig. 2. Schematic of complimentary expertise for preclinical development of influenza virus therapies.

Cross-Project synergy within AD3C. A major strength of this program is that similar developmental approaches will be shared across all the Projects. Active compounds will be cross-tested to identify compounds with broad spectrum antiviral activity. Perhaps the best example is EIDD-1931 and its prodrug that are active against influenza, Chikungunya virus and Venezuelan equine encephalitis (both in Project 2) and coronavirus (Project 1). Progress with this compound in any of these projects will advance the development of this compound across all projects. All other active compounds will also be cross-tested in the other three Projects to help reveal those with the potential to treat more than one virus.

SCIENTIFIC PREMISE. The premise of the proposed research is based on strong precedents, as basic and translational research has generated efficacious clinical treatments against viral infections such as HIV and HCV. Furthermore, our previous work with influenza virus showed the improved efficacy of combination regiments and is also consistent with experience with HIV and HCV. Our lead molecules will be chemically optimized for drug-like properties and broad antiviral activity and tested for *In vitro* and *In vivo* efficacy. A clear progression plan will ensure that we reach our proposed milestones.

EXPERIMENTAL RIGOR AND CONSIDERATION OF RELEVANT BIOLOGICAL VARIABLES. To improve precision, all *in vitro* experiments will be performed using multiple technical and biological replicates. Proper controls including untreated, vehicle only, positive control drugs, and non-functional analogs will be included in each experiment. When possible, viral stocks will be generated from cDNA clones and sequence confirmed. Mouse experiments will be used to assess *in vivo* efficacy of lead molecules against different subtypes of influenza. In the Vertebrate Animals section, we provide sample size estimates based upon power calculations. In general, animal experiments will be done in small batches to ensure reproducibility. Animals will be purchased from commercial vendors to ensure quality and reproducibility. Mice will be randomized into experimental groups to reduce bias and the analysis will be blinded. Equal numbers of male and female mice will be used to monitor for sex-based differences. Reporting of experimental data will be transparent to ensure reproducibility by others. Results will be analyzed by parametric and non-parametric analyses, depending upon data distribution, making appropriate corrections for multiple comparisons.

Aim 1. Identify potent influenza compounds targeting RdRp complex functions

Hypothesis and rationale: We hypothesize that novel molecules identified through recent evaluation of propriety libraries will target functions of the RdRP complex and warrant further development as antiviral therapies for influenza virus infections. Lead molecules were identified in the previous CETR and were selected on the basis of potency, selectivity, replication specificity, and chemical tractability. Some of the hits described

here will enter a lead optimization program and the resultant compounds will be re-characterized for antiviral activity. Lead optimization and retesting for activity/toxicity/bioavailability will be an iterative process, involving multiple rounds of synthesis/biological evaluation. In comparison, other molecules that we have identified appear to be sufficiently promising to advance rapidly into animal models in Aim 3. Our goal is to identify compounds against the conserved functions of influenza virus RdRP complex that might be effective against multiple viral strains of the virus. Compounds that exhibit specific inhibitory effects of RdRp complex functions will undergo hit follow-up and at least limited SAR investigation, PK analysis and toxicity testing in collaboration with scientists at GS and EIDD.

Experimental approach: Active compounds from the EIDD, SR, and GS compound collections will all be evaluated using established and validated assays to ensure that they can be compared directly using the same assays. Additional analogs of these compounds are available and will be tested immediately to determine if medicinal chemistry campaigns are warranted. Preliminary data with these compounds (**Fig. 1 and Table 2**) suggest that they inhibit functions of the RdRp complex by at least two distinct mechanisms; thus, some of the compounds should be advanced into animal studies rapidly. The set of 1000 EIDD compounds also will be immediately evaluated by the IF assay described above to identify other analogs that inhibit the replication of influenza. With this body of data, the medicinal chemistry program will make direct comparisons of efficacy and prioritize their efforts on molecules that warrant iterative synthesis/evaluation. Further characterization of their mechanism of action will be assessed by the assays described in **Aim 2**.

1A. Continue hit to lead optimization of the active compounds from EIDD, GS, and SR. Compounds identified from the completed SR HTS campaign as well as those from EIDD and GS will be prioritized by evaluating analogs from commercial sources and identifying proprietary chemical scaffolds that warrant further testing. The best compounds from this approach as well as active analogs from the GS and EIDD collections, will be evaluated using secondary and tertiary assays in Aim 2 designed to prioritize candidate compounds for medicinal chemistry studies. Novel compounds presented above were selected by methods designed to enrich for inhibitors of RdRp complex function, and preliminary studies are consistent with the hypothesis of this aim. To ensure that the compounds target this complex, we will eliminate hits that target viral entry and those that induce interferon (IFN) as they have proven to be of modest efficacy in the treatment of influenza virus infections. Compounds that prove to be independent of entry and IFN induction will be tested in a viral polymerase assay. The RdRp assays described in Aim 2 will confirm that compounds target the RdRp complex and establish the potency of inhibition. Hit compounds will undergo further development by advancing the best RdRp complex inhibitors into a medicinal chemistry program. One compound, EIDD-1931, is in more advanced development, and data to justify animal studies will likely be assembled more rapidly.

The **Assay Core** is equipped to efficiently identify compounds with activity against influenza virus. The following assays were utilized to identify the active molecules in **Table 2** and will provide a basic outline of assays to be used to help prioritize compounds.

Immunofluorescence (IF) assay. The 892 hits from the ELVIRA® screen in the previous CETR were confirmed in an assay that quantified the surface expression of the M2 protein in A549 cells using a mAb conjugated to Alexa Fluor 647. Total fluorescence intensity was measured with a Mirrorball® fluorimeter. In these assays, cytotoxicity was evaluated in parallel in the same cells using CellTiter-Glo as an endpoint. Concentrations sufficient to inhibit cell viability by 50% (CC_{50}) together with antiviral data described above identified the best molecules by selecting those with selective indices (CC_{50}/EC_{50}) of >10 and EC_{50} values of <15 μM.

Rapid virus titer reduction (VTR) assay. Production of progeny virus will be used as a follow up assay to confirm the potency of the antiviral effects. Briefly, supernatants from infected A549 cells treated with 10 and 1 μM test compounds of interest will be diluted over a six-log range to titrate virus infection in ELVIRA® reporter HEK293 cells. The virus tissue culture infectious dose 50% ($TCID_{50}$) titer will be calculated and compared to that of the virus control to determine the reduction in progeny virus titers associated with compound treatment.

Interferon and attachment assays. Compounds that act through the induction of interferon (IFN) or inhibit virus attachment will be eliminated early in development. IFN levels will be determined with a commercially available sandwich ELISA to test for human IFNα and Jurkat reporter cell lines described in **Project 2**, using calibrated recombinant proteins and drugs (e.g.,) known to induce IFN as controls. To detect compounds that inhibit virus attachment steps, the rapid VTR assay will be repeated using cells that are treated with compound dilutions at 1 h following infection. Virus replication will be determined by RT qPCR and $TCID_{50}$ titration at 24 h following infection.

Perform SAR, lead development and optimization. As described in preliminary studies, we have identified two non-cytotoxic (CC_{50} > 40 μM) hits **SRI-19656** [EC_{50} = 1.32 μM, VTR > 4 logs at 10 μM], and **SRI-36768** [EC_{50}

= 2.55 µM, VTR > 4 logs at 10 µM] that show activity against the H3N2 strain of Influenza A virus (**Table 2**). SRI-19656, also known as Brequinar, is a potent dihydroorotate dehydrogenase inhibitor which has been evaluated in multiple cancer clinical trials (38, 39). Recently, several analogs of **SRI-19656** also were reported as potent H1N1 inhibitors (40). We will begin this sub-aim by purchasing commercial analogs of **SRI-19656** to test in the IF/VTR assay for their antiviral activity. Compounds with an EC_{50} (H3N2) < 15 µM, CC_{50} > 50 µM, and VLR > 3 logs will then be evaluated for antiviral activity against multiple strains of Influenza virus such as H1N1, H5N1 and influenza B using the rapid yield reduction assay (VTR) and the secondary assays such as polymerase assay in Aim 2. Illustrated in **Fig. 3** is our lead optimization and developmental pathway that will be followed to

(b) (4)

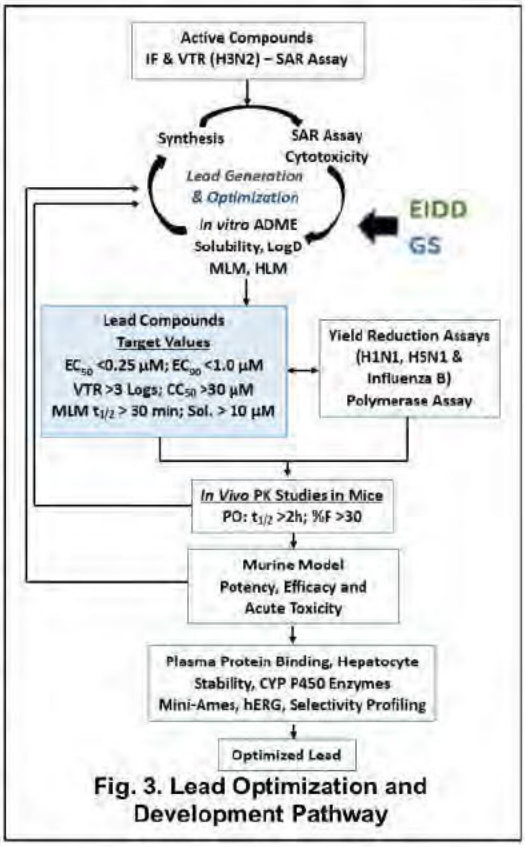


Fig. 3. Lead Optimization and Development Pathway

cases, inactive compounds also will be evaluated in ADME assays. Data of this nature will be used to understand the effect of chemical modifications to improve solubility or microsomal stability, and thus should further guide the design process for the next generation of analogs. Compounds with the desired antiviral activity and acceptable ADME profile will advance to the yield reduction assay and secondary assays for both the influenza A and B pathogen-related potency and spectrum of a compound using pimodivir and baloxavir as controls. Compounds will be prioritized and selected for *in vivo* pharmacokinetics (PK) studies in mice based on activity, potency, and *in vitro* ADME [EC_{90} < 1 µM; CC_{50} > 30 µM; VTR > 3 logs at 10 µM; MLM t½ >60 min; Sol. >10 µM] properties (Aim 3).

The design and synthesis of **SRI-19656** and **SRI-36768** analogs was based on chemical modifications in an effort to improve the activity and drug-like properties. For example, some areas of chemical modifications (A–D) in SRI-36768 are shown in **Fig. 4**. A few sets of compounds at each indicated site will be designed, synthesized and tested for activity and the ADME (solubility and microsomal stability) properties of these analogs will be determined as described in the Product Development Plan. The design of new compounds will follow Lipinski's rule of 5, a rule to evaluate 'drug likeness' as well as calculate drug-like properties of new analogs, such as solubility (LogS), lipophilicity (clogP), and (b) (3) (A), (b) (4) Composite Site Liability (CSL) for metabolically unstable areas in the molecule will be determined. Cytochrome P450 profiles generated by (b) (3) (A), (b) (4) for SRI-36768. These data will help refine the selection of targets going forward and inform critical **Go/No-Go** decisions.

Synthesis of analogs and data management. The synthesis of compounds will occur by considering literature precedents as well as by methods developed by the chemists at SR. It is anticipated that 150 analogs will be synthesized each year, Year 1 through Year 3. During Years 4 and 5, it is expected that compounds with improved ADME profiles will have been identified and less chemistry effort will be required. Approximately 10-25 mg quantities of target compounds will be prepared in >95% purity for SAR studies, and the structure and purity will be confirmed by NMR, Mass Spectrometry and HPLC. Gram quantities of selected compounds for animal experiments will be synthesized when necessary. An informatics and compound registration database, (b) (3) (A), (b) (4), at SR is used for data capture, analysis and storage of biological activity and ADME and PK data on all newly synthesized compounds.

(b) (4)

***In vivo* Absorption, Distribution, Metabolism and Excretion (ADME).** The **MCLDC** will coordinate all *in vivo* PK studies of selected compounds using standard well-established protocols at Pharmaron Inc (**Aim 3**). A total of 6-8 compounds will be nominated with acceptable *In vitro* ADME properties, antiviral potency and efficacy for *in vivo* PK studies. The PK profile of a compound will be assessed in male CD1 mice following single PO administration (e.g., dose: 5 mg/kg, vehicle: 2% DMSO/3% solutol in water). Properties, such as plasma half-life ($t_{1/2}$), peak plasma concentration (C_{max}), time to reach C_{max} (T_{max}), systemically available fraction of a compound (bioavailability %F), clearance (CL), volume of distribute (V_d) and area under the curve (AUC_{inf} and AUC_{last}) will be determined. Compound dose and frequency of administration for *In vivo* efficacy studies in murine models will be calculated based on these PK properties. If acceptable exposure of compound is not achieved by the above said formulation, other formulations will be evaluated. In the absence of acceptable exposure ($t_{1/2}$ > 2h and bioavailability F > 30%), other routes of administration, such as IV, SC and IP for these animal studies will be investigated.

Off-Target Interactions: Compounds with acceptable PK profiles (bioavailability F > 30%, half-life > 2 h) and *In vivo* efficacy will be analyzed for off-target interactions. For example, assessment of the potential of a compound to inhibit a specific cytochrome P450 enzyme, the major enzymes of primary metabolism in the liver, is important as its administration may inhibit its metabolism, or that of other co-administered drugs. This may affect plasma levels *in vivo* and potentially lead to adverse drug reactions or toxicity. Specifically, the six major drug metabolizing CYP450s in humans will be tested: CYP1A2, CYP3A, CYP2D6, CYP2C9, CYP2C19, and CYP2E1. Cultured mouse or human hepatocytes will be incubated with 1 μM of test compound for 2 h, whereupon the supernatant analyzed by LC/MS/MS. Control reactions in the absence of compound and with heat-inactivated hepatocytes will be included to facilitate detection of compound metabolites over normal cell metabolites. While the MS/MS data will help identify the type of metabolic transformation (e.g., hydroxylation of a particular aromatic ring), the exact structure of the metabolite (e.g., location on the ring) will not be definitively determined. Nevertheless, this information will suggest specific sites of modification based on literature precedent and chemical logic. These approaches will be tested through the synthesis of compounds designed to block metabolism at the proposed sites. The compounds also will be tested in a high-throughput panel of assays for effects toward off targets (e.g. Panlabs, 240 host derived targets at 10 μM compound concentration) in which a wide range of target classes are evaluated, including GPCRs, kinases and nuclear receptors.

Expected Outcomes. We will identify at least one primary and one back-up compound to be tested for further *in vitro* and *in vivo* efficacy in **Aims 2 & 3**. The back-up compound will be structurally different than the primary compound. As with all of SR's drug discovery programs, continuous evaluation of progress and timelines will occur and realistic **Go/No-go** decisions will be made as the described medicinal chemistry strategies are executed. As part of the lead optimization, multiple parameters are considered, including novelty, synthetic feasibility, off-target liabilities and toxicity.

Potential Problems and Alternate Strategies. The strategies outlined here should lead to novel inhibitors of Influenza virus that have acceptable drug-like properties, such as solubility and metabolic stability. It is common that optimization for one parameter may negatively impact another parameter, and various modifications will need to be evaluated to achieve a balance between the desired potency and preferred drug-like properties. We are cognizant of this realty and will optimize efficiently and effectively, using our iterative medicinal chemistry and PK knowledge to put forth the best inhibitor(s) for subsequent development. If any off-target interactions are observed, as an example hERG, the back-up series can be advanced or analogs will be designed and synthesized focusing on the identified issues. This would include preparing compounds where the physiochemical properties have been changed, adding an acid moiety, increasing oxygen H-bond acceptor and modifying the core/linkers to (41, 42). Of course, it is possible that compounds or chemical series cannot be optimized for acceptable drug-like properties with the preferred biological activity and delivery by inhalation or the parenteral route would also be considered. We will continuously make **Go/No-go** decisions as we execute lead optimization. As part of this process, we will discuss issues or concerns with the Administrative Core and Executive Committee and Scientific Advisory Committee.

Aim 2: Define the mechanism of action and spectrum of antiviral activity of lead compounds

Hypothesis and rationale: We hypothesize that many of the compounds identified through studies in Aim 1 will inhibit functions of the RdRp complex and preliminary data presented here appear to confirm this. In this Aim, hit compounds will be evaluated against a panel of H3N2, H1N1, B, H5N1, HPAI strains and drug-resistant strains to define their spectrum of antiviral activity using pimodivir and baloxavir as controls. Representatives of the best chemical scaffolds identified in **Aim 1** will be characterized further to confirm that the compounds inhibit functions of the RdRp and are also active against strains such as H7N9. The molecular targets of lead chemical

scaffolds will be identified through the generation of resistant isolates followed by genetic engineering of mutations into a laboratory strain of the virus. Combination studies with lead compounds and approved inhibitors of influenza virus will be performed to assess synergy and the prevention of resistance. Finally, lead broad-spectrum antiviral compounds derived from the other three Projects will be evaluated against each of the influenza virus subtypes.

Experimental approach: The compounds identified in the preliminary studies will be further evaluated using secondary and tertiary assays designed to prioritize the best chemical scaffolds based on criteria outlined in Aim 1. To focus on the hit compounds affecting RdRp complex functions we will focus on those that are active against all subtypes of influenza and in assays for RdRp function. This includes evaluation of viral RNA synthesis, a transient cell-based minigenome assay, a cell-based cap snatching assay, and enzymatic assays. For lead compounds the kinetics and nature of viral escape mutations will be characterized by serial passage in the presence of the compounds and sequencing of resistant isolates. Specific mutations will be engineered into the PR8 strain to formally ascribe them to the resistance phenotype. The mutant viruses will be evaluated for replicative fitness and for potential cross resistance to other approved RdRp inhibitors.

2A. Define spectrum of antiviral activity against wild-type, AIV, and NAI resistant strains of influenza virus. Hit compounds that have been confirmed to act by a post-entry mechanism of action in Aim 1 will be tested for activity in a set of influenza virus subtypes and resistant isolates including H1N1 A/Hawaii/21/2007 (NA-H274Y mutant), H3N2 A/Wisconsin/67/2005 (S31N), and B/Florida/4/2006. Lead compounds will also be evaluated against AIVs to ensure they maintain activity against this strain. Compounds also will be tested against oseltamivir resistant isolates to ensure that they retain efficacy.

Virus Yield Reduction (VYR) Assay: Standard VYR Assays will be performed by infecting A549 cells with each influenza virus strain and progeny virus resulting from the infection will be titrated in 384-well plates containing monolayers of MDCK cells. Virus CPE will then be detected with CellTiter-Glo $TCID_{50}$ titers will be determined. This assay is robust and has proven to be useful for all strains of influenza virus tested to date including resistant isolates and will include a ribavirin positive control in each assay.

Highly pathogenic avian influenza (HPAI) strains: MDCK cells will be infected with the HPAI A/VN/1203/04 isolate of influenza virus. Infected cells will then be seeded into 384-well plates containing test compounds by methods described above for the rapid VTR assay and will include the same ribavirin control compound. Cell viability will be determined at 72 h following infection using Cell Titer-Glo. Supernatants will also be retained for a rapid VTR assays. Subsequently, other HPAI strains available at SR (H7N9 A/Anhui/1/2013, A/Indonesia/5/2005 H5N1, and A/Viet Nam/1203/2004 H5N1) and LPAI strains can also be tested.

2B. Characterize the mechanism of action (MOA) against RdRp functions. Active compounds described in Aim 1 that also exhibit antiviral activity against other subtypes of influenza will be tested to define their effect on functions of the RdRp complex.

Inhibition of RNA synthesis. To detect compounds that interfere with the synthesis of influenza RNA synthesis, A549 cells will be infected at an MOI of 0.5 and dilutions of test compounds will be added at 1 h following infection. Total RNA will be harvested, and RNA will be quantified using Taqman Fast Virus 1-Step Master Mix (Applied Biosystems) with T-705 and ribavirin included as positive control drugs.

Minigenome assay. A549 cells will be transiently co-transfected with the influenza virus firefly luciferase mini-genome reporter, pRL-TK control reporter, PB1, PB2, PA and NP protein encoding plasmids. Following a 24 h incubation, the transfected cells will be lysed and luciferase production measured with the Dual-Glo luciferase assay kit (Promega). As negative control, A549 cells will be transfected as above except for the NP plasmid; an empty vector plasmid will be used instead of NP. The control compound, ribavirin, will be included in each experiment to confirm that the assay is functioning correctly.

Cell based cap snatching assay and polyadenylation assay. We designed a qPCR assay to quantify specific cap snatched mRNA molecules that contain the U2 spliceosome cap and the PB2 coding region (30). Primers specific for the junction of the U2 snatched RNA and the PB2 gene were designed with a forward primer (host U2 nucleotides underlined) 5'-ATA CTT ACC TGG CGA AAG-3', reverse primer 5'-CAT ATG GTC CAC GGT GGT TT-3', and probe 5'-FAM-AAT CTA ATG TCG CAG TCT CGC ACC C -BHQ-3'. Briefly, monolayers of A549 cells in 96-well plates will be infected with the PR8 strain of influenza virus (43), compound dilutions added at 1 h following infection, and total RNA will be isolated as described for the RNA synthesis assay above. Resulting RNA will then be reverse transcribed with a high capacity RNA-to-cDNA kit (Thermo Fisher Scientific), and mRNA levels will be quantified using the primers described above. Levels of polyadenylated transcripts in PR8 infected cells will be determined on poly-A selected cDNAs using favipiravir and pimodivir as controls.

***In vitro* cap snatching assay.** An *In vitro* assay will utilize synthetic capped RNA and NTPs mixed with the RdRp-viral RNA (vRNA) complex and levels of chimeric cap snatched mRNAs will determined. Synthetic capped-RNA will be generated that corresponds to the 5´ half (102 bases) of human U2 small nuclear RNA (U2-snRNA) (30). *In vitro* transcription and capping will be performed using the mMESSAGE mMACHINE® Kit (Thermo Fisher Scientific), to synthesize capped RNA. The RdRp complex will be isolated from influenza A viral particles using conditions that expose the RdRp-vRNA complex or a recombinant RdRp-vRNA complex (44). Cap snatching and polymerization detection will use either an alpha screen with streptavidin beads and anti digoxigenin acceptor beads, or with a digoxigenin-labeled probe. While the alpha screen approach would be rapid, the probe approach is expected to be more sensitive since the labeled nucleotide is incorporated all along the polymerized sequence. Controls will include T-705 and VX-787 in each assay.

***In vitro* endonuclease assay.** Specific inhibition of the endonuclease activity of the purified PA N-terminal domain (PA N-ter) will be measured using a TaqMan probe as a substrate for the endonuclease. Synthetic ssDNA substrates are cleaved with only slightly reduced activity compared to RNA (45) and will contain the 5´-AGCA-3´ motif required for endonuclease activity (46). Cleavage of the synthetic probe will separate the fluorescent tag from the quencher and result in a detectable fluorescent signal. Chelating compounds will be counter selected in a parallel assay using a double-stranded (ds) DNA TaqMan probe containing a restriction site for a type-II restriction enzyme**.** This class of enzymes has structural similarity with PA N-ter domain and also require divalent cations (Mn^{2+}, Mg^{2+}) for activity, showing sensitivity to chelating agents (45, 47). Controls will include baloxavir (positive) and ribavirin (negative).

PB1-driven RNA elongation of purified vRNP. The *de novo* RNA synthesis activity catalyzed by PB1 will be evaluated using a dimeric form of PA with PB1 (residues 1-686) shown to be functional in RNA synthesis in absence of capped mRNA or ApG. Purified PA-PB1(1-686)) polymerase will be incubated with an 80-mer panhandle RNA (48), NTPs and dig-11UTP. Dig-labelled polymerization products will be detected using an anti-digoxigenin-HRP and a chemiluminescence substrate as in the previous section.

Cap binding assay. This assay will consist of measuring the molecular interaction between PB2 and the same synthetic capped-RNA described in the previous section. For this experiment we will use a bio-layer interferometry (BLI)-based assay. The capped-RNA will be 3´ biotinylated and bound to a streptavidin coated probe. The middle PB2 cap-binding domain (residues 318-486) will be used to measure binding to the capped-RNA. Any compound interfering with PB2 binding can be detected by the change in the wavelength of reflected light. SR has expertise in BLI studies and a state-of-the-art BLI ForteBio Octet machine.

2C. Map molecular determinants of resistance and their impact on susceptibility and replication fitness. Molecular determinants of resistance will be identified for the lead molecules that target the RdRp, or for inhibitory compounds that do not appear to be active in the specific assays for the RdRp (i.e., have another mechanism of action). Resistant isolates will be selected using the cloned PR8 strain by methods reported previously (43, 49). Briefly, the virus will be passaged in increasing concentrations of the test compound starting at the EC_{50} and escalating by two-fold with each passage. If insufficient quantities of progeny virus are obtained at some passages, the virus can be amplified in the absence of the compound or the selection can continue at a reduced concentration. Infected monolayers will be inspected daily, and the virus will be harvested when significant cytopathology is observed or 10 d following infection. The isolate will be sequenced at the UAB Heflin Center for Genomic Science NexGen facility to identify substitutions using primers described previously (50). Resistance can also be confirmed in a standard CPE reduction assays using favipiravir, pimodivir, and baloxavir to evaluate PB1, PB2, and PA cross resistance, respectively. Kinetics of resistance will be determined by sequencing retained samples from each passage to test for the resistance mutation and compared to the kinetics of resistance of a parallel selection of oseltamivir resistance used as a control.

Identified substitutions will be engineered into the PR8 plasmid clone by site directed mutagenesis (Quick change II mutagenesis kit, Agilent). The mutagenized plasmid together with the other seven plasmids will be transfected into of 293T cells and aliquots of the supernatant will be transferred to MDCK cells to propagate the resulting virus. Sequencing of each segment will confirm that the desired mutation is present and that no extraneous mutations were introduced. Resistance will of the mutants will be confirmed in *in vitro* assays and cross resistance with other RdRp inhibitors will also be evaluated.

Decreased replicative fitness of resistant isolates is anticipated as mutations in essential genes are typically deleterious. Competition studies with resistant isolates and the parent virus with molecular tags will detect replication defects using qPCR as an endpoint. Cross resistance with other RdRp inhibitors will also be tested.

2D. Perform combination studies and evaluate lead molecules from other three Projects. The efficacy of the best compounds will be evaluated in combination with oseltamivir as well as baloxavir, pimodivir, and

favipiravir by methods reported previously (51). Briefly, a checkerboard matrix of compound concentrations will be tested in infected A549 cells and levels of viral RNA will be used as an endpoint to quantify virus replication. Levels of viral RNA will then be used to calculate quantify potential synergistic interactions by methods we reported recently (52). These studies are important because combination therapy likely will be required to treat influenza virus infections, and data will help to justify doses for *in vivo* combination studies. These studies are important as combinations of drugs against distinct molecular targets have proven to me tremendously effective against HIV and HCV infections. Antiviral activity of lead compounds from other projects will also be evaluated using assays described in Aim 2A.

Expected outcomes: We anticipate that lead compounds from the SR, EIDD, and GS collections will target essential functions of the RdRp complex based on chemical structure, preliminary data presented here, and studies reported for other viruses (25). Biochemical assays as well as reverse genetic techniques are expected to define specifically the molecular targets of these compounds in influenza virus.

Pitfalls: We anticipate no difficulties with established antiviral assays. However, difficulties may arise with enzymatic assays, potentially related to the specific mechanism of action of uncharacterized lead compounds. Alternative assays are available if hurdles arise in the determination of specific biochemical defects for a particular drug. Generation of resistant isolates might be problematic for some compounds, particularly nucleoside compounds that are incorporated into RNA. If the amplification and sequencing prove to be inefficient, the resistant isolate will be passaged a single time without the compound in a high MOI infection to produce additional RNA without incorporated test compounds.

Aim 3: Evaluate compounds in mouse models of influenza.

Hypothesis and rationale: Compounds meeting the potency and bioavailability criteria defined in **Aim 1**, (*in vitro* potency (EC_{90} < 1 μM), selectivity (SI > 100 in human cells), and acceptable bioavailability (mouse plasma levels ≥ 5 fold EC_{50} by IP administration, and adequate pharmacokinetic parameters), will be evaluated using in-house murine infection models at SR (53). Additional rounds of chemical optimization are expected after the murine studies. Potent and non-toxic compounds showing efficacy against H1N1 influenza in mice will be further tested in AIV strains in mice and in ferret models at SR in the final year of the grant.

Experimental approach: MCLDC will be responsible for all phases of the optimization, scale-up, and submission for *in vivo* testing in this aim. All newly synthesized compounds will be fully characterized using standard spectroscopic and chromatographic tools (HPLC, LC/MS, NMR, MS, and elemental analysis) as well as other measures recommended by EIDD and GS. Animal studies will commence in Year 2 starting with maximal tolerated dose (MTD) and PK studies for the most advanced compounds. Initial efficacy studies for compounds meeting acceptability criteria discussed above will also take place starting in Year 2 and continuing through year 3. In Years 4 and 5, murine efficacy studies will continue, and HPAI studies will be initiated. Also in this year, with an optimal lead the first ferret study will be initiated with a second scheduled in Year 5 of the grant.

3A. Perform maximum tolerated dose (MTD) and pharmacokinetic (PK) studies in mice. The MTD and PK parameters will be determined at SR on the best chemical scaffolds prior to efficacy studies. Studies will be designed with the fewest number of animals possible, consistent with the objective of the study to determine the MTD of a novel therapeutic. Briefly, 5 groups of 10 animals each with doses of 30, 10, 3, and 1 mg/kg and a vehicle control will be dosed once daily for 14 d and examined twice daily for clinical signs including, but not limited to, activity, hunched posture, poor grooming, anorexia, dyspnea, squinting, nasal and ocular discharge, neurological signs, or other abnormalities. PK studies will also be done using standard well established protocols at Pharmaron Inc. with all animals fed prior to dosing. PK calculations will estimate the $T_{1/2}$, C_{max}, T_{max}, AUC_{inf}, AUC_{last}, and $F_{(0-\infty)}$ %. Each mouse will be given a single dose of test compound and the control group will be given vehicle only. Animals will be observed and weighed, and plasma tissue will be collected from each group that are euthanized at 15 min, 30 min, 1, 2, 4, 8 and 24 hr. Samples will be analyzed by HPLC-MS/MS to determine the concentrations of intact test compound and metabolites of the compound, as appropriate.

3B. *Evaluate efficacy against mouse-adapted A/California/04/2009 (H1N1) infection in mice.* Initial, dose ranging studies will determine the MTD, and compounds with acceptable bioavailability and pharmacokinetic parameters (bioavailability F > 30%, half-life > 2 h) will be evaluated further in efficacy studies.

Prophylactic therapeutic regimen: A basic study outline for *in vivo* efficacy studies in mice will utilize A/California/04/2009(H1N1) mouse adapted influenza virus strain administered by intranasal challenge of male and female BALB/c mice, which have been successfully used in pathogenicity studies for this virus (53). The study was designed to use the fewest number of animals possible, to detect significant efficacy of a novel therapeutic. The test articles will be administered (in DPBS or medium determined by compound solubility), using the method evaluated for dose study. To obtain groups that are comparable by body weight, all mice will be

assigned randomly by weight to one of six groups (**Table 4**). On Day 0 (4 h, ± 30 min, prior to challenge), mice will be administered the appropriate test article. Animals in Groups 1 and 3 will have test article administered at 4 h (± 30 min) prior to virus challenge on Day 0. Groups 2, 4, 5, and 6 will have test article administered on Day 0, 4 h (± 30 min) prior to virus challenge and then daily on Days 1-4 24 h (± 30 m post the previous test article/vehicle administration). Also on Day 0, mice will be anesthetized and challenged with the virus, or vehicle. If the compounds prove to be active, delayed treatment could also be tested. Retained challenge inoculum will be titered to confirm the infectious dose instilled.

Table 4. Design of in vivo efficacy studies in mice

Dose Group	Test Article	Dose Level (mg/kg/day)	Challenge Material	Challenge Dose	Challenge Volume (µl)	Number of Mice
1	Compound	TBD[a]	H1N1	10 LD_{50}	90	10
2	Compound	TBD	H1N1	10 LD_{50}	90	10
3	Compound	TBD	DBPS	---	90	10
4	Compound	TBD	DPBS	---	90	10
5	Compound	Vehicle[b]	H1N1	10 LD_{50}	90	10
6	Control	Oseltamivir (50)	H1N1	10 LD50	90	10

[a] To be determined.
[b] Vehicle will be DPBS and serve as the virus control group.

Evaluation of data: All mice will be observed at least twice daily throughout the pre-study and study periods for signs of morbidity and mortality. Clinical signs will be observed daily from Day -1 through Day 14 and will include, but not be limited to, activity, hunched posture, poor grooming, anorexia, dyspnea, squinting, nasal and ocular discharge, neurological signs, or other abnormalities. Body weights for all animals will be obtained during Week -1 (randomization) and daily from Day 1-14 (prior to dosing on Days 0-4), or more often as warranted. Body temperatures for all animals will be obtained daily on Day 1-14 (prior to dosing on Days 0-4). Animals that become moribund or have a body weight loss that equals or exceeds 30% will be euthanized via CO_2 inhalation. All remaining animals will be euthanized on Day 14 via CO_2 inhalation. Group means and standard deviations will be calculated for body weights, temperatures, and any other data deemed appropriate. Virus titers in lung tissue will also be evaluated on Day 14 to confirm that the animals had cleared the infection.

3C. Characterize efficacy of lead compound(s) in ferrets infected with A/California/04/2009 (H1N1). After the mouse studies have been completed and lead compounds will be evaluated in a ferret model. The ferret model is the "gold standard" for testing therapeutics and vaccines against influenza strains as these animals are readily infected with human strains and follow a similar disease progression as humans (54).

Fitch ferrets will be purchased from a qualified vendor and prescreened by hemagglutination inhibition (HAI) assay to eliminate those with previous exposure to circulating seasonal influenza virus or the homologous strain used for challenge. The study design will be similar to that in **Table 4** with the following differences. A total of 36 ferrets will be randomized by weight into six groups of 6 animals each and either treated prior to, or after viral challenge. On Day 0, animals will be anesthetized and challenged by intranasal administration of A/California/07/2009(H1N1). The infectious dose will be increased to 1 x 10^6 $TCID_{50}$ units in 1 ml, which has been shown to generate moderate to severe infection. At 4 h (± 30 min) post challenge, all ferrets will be initially treated with the test compound. Animals in Groups 1 and 3 will receive no additional doses after the initial treatment. Groups 2, 4, 5 and 6 will have test article administered daily on Days 1 – 4 (24 h ± 30 min between doses) post challenge and after the initial treatment. Delayed treatment studies could also be considered for highly active compounds. Challenge inoculum will be retained and titrated to confirm the infectious dose instilled.

After challenge, animals will be monitored daily for 14 D for disease progression using clinical signs, including body weights, temperature and activity scores, and to identify moribund animals. Animals declared moribund will be promptly euthanized and removed from study. In addition, nasal wash procedures will be performed to determine the level of viral shedding on days 1, 3, and 5 post challenge using a $TCID_{50}$ assay. A comprehensive analysis of the end point data between dosing regimens (single versus multiple doses) will be performed using both parametric and non-parametric methods to determine the overall effectiveness of the test compound.

COOPERATIVE PLAN AND RELATIONSHIP TO CETR (AD3C). Our multidisciplinary team includes medicinal chemists, structural biologists, computational biologists, and virologists as well as scientists with pharmaceutical company experience. This team provides cutting edge technologies to develop lead molecules. Interactions with other projects will facilitate the testing of each project's lead compounds against divergent viruses. Together with SR, GS, and EIDD, our AD_3C project is partnered with strong leaders in drug discovery and industry partners to help with translational application and product development.

PHS Human Subjects and Clinical Trials Information

OMB Number: 0925-0001 and 0925-0002

Expiration Date: 03/31/2020

Are Human Subjects Involved	○ Yes ● No
Is the Project Exempt from Federal regulations?	○ Yes ○ No
Exemption Number	❑ 1 ❑ 2 ❑ 3 ❑ 4 ❑ 5 ❑ 6 ❑ 7 ❑ 8
Does the proposed research involve human specimens and/or data	○ Yes ● No
Other Requested information	

Description of Procedures

These will not be GLP studies.

1- Perform maximum tolerated dose (MTD) in mice

Objective: The objective of this study is to determine the tolerance of BALB/c mice to 14 consecutive days of oral gavage (PO) dosing with test compound.

Quarantine: 7 days
Species: BALB/c mice
Supplier: Charles River or other approved vendor
Age: 8 to 10 weeks old
Sex: Equally represented male & female
Total number of animals to be used: 50 per compound. Total TBD depending on number of compounds to be selected from medicinal chemistry studies of Aims 1 and 2 (estimated to be not higher than 10 compounds).

Vehicle:

Name: 20%NMP/40% PEG 400/40% saline
Supplier: Commercial manufacturers
Characterization: Documentation of characterization of the components of the vehicle will be obtained from the Certificates of Analysis. The methods of synthesis, fabrication, or derivation are available through the manufacturers.
Stability & Storage: As specified by manufacturers

Dose Formulation:

Preparation: The dose formulation of compound will be prepared in vehicle to contain nominal concentrations of compound of 1 and 3 mg/mL for PO dosing. For preparation, a weighed amount of compound will be dissolved with the appropriate volume of NMP with vortexing and sonification as needed to obtain a solution. The appropriate volume of PEG 400 will be added and mixed by vortexing. Then, appropriate volume of saline will be added and mixed by vortexing and sonication as needed to obtain a solution or acceptable suspension of 1 and 3 mg/mL.
Handling: Room temperature
Stability: The dose formulation will be prepared on each day of use and considered stable through use for dosing.
Disposition: Unused dose formulation will be discarded after dosing.

Experimental Design:

Group assignment and animal identification: The animals will be assigned to their respective treatment groups using a randomization procedure that respect equal representation of both sexes. Each animal will be given an identification number for this study by ear punch.

Group	Test Article	Dose (mg/kg)	Dose Conc. (mg/mL)	Dose Volume(mL/kg)	DoseRoute/ Treatment Days	No. of Animals
1	Vehicle	0 (vehicle control)	0	10	PO/1-14	10
2	Compound	1	0.1	10	PO/1-14	10
3	Compound	3	0.3	10	PO/1-14	10
4	Compound	10	1	10	PO/1-14	10
5	Compound	30	3	10	PO/1-14	10

Dosing: Days 1-14, on the last day of treatment the dosing will be timed for each animal for blood and tissue collections.

Dose Route: PO: Necropsy any animal that dies for evidence of PO dosing trauma.
Dose Volume: 10 mL/kg
Clinical Signs: Animals will be examined twice daily for clinical signs including, but not be limited to, activity, hunched posture, poor grooming, anorexia, dyspnea, squinting, nasal and ocular discharge, neurological signs, or other abnormalities.
Body Weights: Day 1 prior to dosing, then daily until dosing is complete.
Euthanasia: Animals which are found moribund (>20% body weight reduction) and those that survive until study termination will be euthanized and carcasses discarded without further evaluation.

2- Determine efficacy of compounds against influenza virus infection in mice

Objective: To determine the efficacy of test compounds against influenza virus infection in BALB/c mice.
Quarantine: 7 days
Species: BALB/c mice
Supplier: Charles River or other approved vendor
Age: 8 to 10 weeks old
Sex: Equally represented male & female
Challenge Material: IAV A/California/07/2009 (H1N1pdm)
Test article/dose: TBD
Vehicle: DPBS
Total number of animals to be used: 60 animals per test compound. Total TBD depending on number of compounds to be selected from the MTD and PK studies (estimated to be not higher than 6 compounds).

Experimental Design:

Group assignment and animal identification:

To obtain groups that are comparable by body weight, all mice will be assigned to their respective treatment groups using a randomization procedure that respect equal representation of both sexes. Each animal will be given an identification number for this study by ear punch. After randomization, animals will be assigned to one of six groups as indicated in the table below:

Design of *in vivo* efficacy studies in mice

Dose Group	Test Article	Dose Level (mg/kg/day)	Challenge Material	Challenge Dose	Challenge Volume (µl)	Number of Mice
1	Compound	TBD[a]	H1N1	10 LD_{50}	90	10
2	Compound	TBD	H1N1	10 LD_{50}	90	10
3	Compound	TBD	DBPS	---	90	10
4	Compound	TBD	DPBS	---	90	10
5	Compound	Vehicle[b]	H1N1	10 LD_{50}	90	10
6	Control	Oseltamivir (50)	H1N1	10 LD50	90	10

[a] To be determined.
[b] Vehicle will be DPBS and serve as the virus control group.

Dosing: On Day 0 (4 h, ± 30 min, prior to challenge), mice will be administered the appropriate test article. Animals in Groups 1 and 3 will have test article administered at 4h (± 30 min) prior to virus challenge on Day 0. Groups 2, 4, 5, and 6 will have test article administered on Day 0, 4h (± 30 minutes) prior to virus challenge and then daily on Days 1-4 24 h (± 30 m post the previous test article/vehicle administration).
Dose Route: PO: Necropsy any animal that dies for evidence of PO dosing trauma.
Challenge: On Day 0, mice will be anesthetized and challenged intranasally with H1N1 (mouse-adapted A/California/04/09) or DPBS, as indicated in the group assignment table above. Challenge inoculum will retained and titrated to confirm the infectious dose instilled.
Dose Volume: 10 mL/kg

Clinical Signs: All mice will be observed at least twice daily throughout the pre-study and study periods for signs of moribundity and mortality. Clinical signs will be observed daily from Day -1 through Day 14 and will include, but not be limited to, activity, hunched posture, poor grooming, anorexia, dyspnea, squinting, nasal and ocular discharge, neurological signs, or other abnormalities.

Body Weights: Body weights for all animals will be obtained during Week -1 (randomization) and daily from Day -1 through Day 14 (prior to dosing on Days 0-4), or more often as warranted.

Body temperatures: Temperatures for all animals will be obtained daily on Day -1 through Day 14 (prior to dosing on Days 0-4).

Euthanasia: Animals which are found moribund (>20% body weight reduction) and those that survive until study termination will be euthanized and carcasses discarded without further evaluation.

Group means and standard deviations will be calculated for body weights, temperatures, and any other data deemed appropriate.

3- Evaluate lead compound(s) in ferrets.

Objective: To determine the efficacy of lead compounds against influenza virus infection in Fitch ferrets.
Purpose: To compare the IAV variants to the parent virus strain by intranasal challenge in ferrets.
Quarantine: 7 days, then animals will be prescreened by hemagglutination inhibition (HI) assay for previous exposure to circulating seasonal influenza virus and the homologous strain used for challenge. Only naïve ferrets will be used in this study.
Species: Fitch Ferrets (*Mustela putorius furo*)
Source: Triple F Farms, Inc.
Age: 5 to 8 months old
Sex: Males and females equally represented
Challenge Material: IAV A/California/07/2009 (H1N1pdm)
Test article/dose: TBD
Vehicle: DPBS
Total number of animals to be used: 60 animals per test compound. Total TBD depending on number of compounds selected from the mice studies (estimated to be not higher than 4 compounds).

Experimental Design:

Group assignment and animal identification:

Ferrets will be assigned to their respective treatment groups using a randomization procedure that respect equal representation of both sexes. Each animal will be given an identification number for this study by ear punch. After randomization, animals will be assigned to one of six groups as indicated in the table below:

Design of *in vivo* efficacy studies in ferrets

Dose Group	Test Article	Dose Level (mg/kg/day)	Challenge Material	Challenge Dose	Challenge Volume (µl)	Number of ferrets
1	Compound	TBD[a]	H1N1	10^6 $TCID_{50}$	1000	6
2	Compound	TBD	H1N1	10^6 $TCID_{50}$	1000	6
3	Compound	TBD	DBPS	---	1000	6
4	Compound	TBD	DPBS	---	1000	6
5	Compound	Vehicle[b]	H1N1	10^6 $TCID_{50}$	1000	6
6	Control	Oseltamivir (50)	H1N1	10^6 $TCID_{50}$	1000	6

[a] To be determined.
[b] Vehicle will be DPBS and serve as the virus control group.

Dosing: At 4h (±30 min) post challenge, all ferrets will be initially treated with the test compound.

Animals in Groups 1 and 3 will receive no additional doses after the initial treatment. Groups 2, 4, 5 and 6 will have test article administered daily on Days 1 – 4 (24h ±30 min between doses) post challenge and after the initial treatment.

Dose Route: PO: Necropsy any animal that dies for evidence of PO dosing trauma.

Challenge: On Day 0, animals will be anesthetized and challenged intranasally with challenge IAV strain or DPBS, as indicated in the group assignment table above. Challenge inoculum will retained and titrated to confirm the infectious dose instilled.

Dose Volume: 10 mL/kg

Clinical Signs: After challenge, animals will be monitored daily for 14 days for disease progression using clinical signs, including body weights, temperature and activity scores, and to identify moribund animals. Moribund criteria include; Greater than 20% body weight loss compared to pre-challenge weight, neurological deficits, respiratory distress and non-responsive to external stimuli.

Body Weights: Body weights for all animals will be obtained during Week -1 (randomization) and daily from Day -1 through Day 14 (prior to dosing on Days 0-4), or more often as warranted.

Body temperatures: Temperatures for all animals will be obtained daily on Day -1 through Day 14 (prior to dosing on Days 0-4).

Euthanasia: Animals which are found moribund (>20% body weight reduction) and those that survive until study termination will be euthanized and carcasses discarded without further evaluation.

In addition, nasal wash procedures will be performed to determine the level of viral shedding on days 1, 3, and 5 post challenge using a $TCID_{50}$ assay. A comprehensive analysis of the end point data between dosing regimens (single versus multiple doses) will be performed to determine the overall effectiveness of the test compound to attenuate either infection or clinical signs associated with H1N1 influenza virus infection. Group means and standard deviations will be calculated for body weights, temperatures, and any other data deemed appropriate.

Justifications

There is no alternative to animal use for these types of toxicity and efficacy experiments. We have designed this experiment with minimum number of animals providing the maximum results. It is the policy of Southern Research that all research involving animals be conducted according to the highest possible professional and ethical standards. The Institutional Animal Care and Use Committee (IACUC) of Southern Research provides the guide for animal care and use for our program. Our Public Health Service (PHS) Assurance Number is A3046-01, and our United States Department of Agriculture (USDA) permit number is 64-R-0001. We are fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care - International (AAALAC; number 000643).

Minimization of Pain and Distress

Maintenance and care of animals is in accordance with the American Association for Accreditation of Laboratory Animal Care standards and will meet or exceed requirements established in the *Guide for the Care and Use of Laboratory Animals* (2011). We will make all efforts needed to minimize discomfort to the animals. The Principal Investigator, laboratory personnel and animal technicians involved in any way in the proposed research have had instruction and have demonstrated their competence in the care, use and handling of laboratory animals. We will ensure all personnel who handle the animals are trained in appropriate animal handling techniques to minimize pain and discomfort. Infected ferrets are monitored for lethargy in their cage and animals showing signs of distress as determined by lack of ability to obtain food or water, lack of response to external stimuli or greater than 20% weight loss will be painlessly euthanized. Discomfort, distress, pain, and injury will be minimized through the use of anesthetics (e.g. ketamine/xylazine, isoflurane) prior to intranasal viral inoculation.

Mice will be euthanized when required according to the Southern Research IACUC standard operating procedures, consistent with the recommendations of the American Veterinary Medical Association (AVMA) Guidelines on Euthanasia.

1. Select Agent to be used in the proposed research: Highly pathogenic avian influenza (HPAI) strain A/Viet Nam/1203/2004 (H5N1) will be used in this project to define spectrum of antiviral activity and for efficacy testing in mouse model.

2. Registration status of all entities where Select Agent(s) will be used. Studies involving select agents for this project (stated above) will be contained to Southern Research's Biosafety Level (BSL)-3 Facilities located (b) (3) (A), (b) (6)

(b) (3) (A), (b) (6) Southern Research is registered with the CDC as well as USDA for the use/storage of Tier 1 and other agents that require BSL-3 containment. The BSL-3 facility's registration number is C20160406-1792, and expiration date is April 06, 2019.

3. Procedures that will be used to monitor possession, use and transfer of Select Agent(s). It is the goal of Southern Research, as a Tier1 BLS-3 facility, to ensure that the A/BSL-3 laboratory is managed and operated in a manner that ensures compliance and/or consistency of best practice with the following:

- NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines), current edition;
- 7 C.F.R. Part 331; 9 C.F.R. Part 121; and 42 C.F.R. Part 73 42
- OSHA Standards 1910 29 CFR 1910; EPA Requirements 40 CFR
- Guide for Care and Use of Laboratory Animals
- 21 CFR Part 58 & Part 11 where GLP work is being conducted
- Biosafety in Microbiological and Biomedical Laboratories (BMBL), current edition
- All applicable state and local regulations

All infectious materials, including select agent materials, are tracked via controlled electronic databases (e.g. Freezerworks) as well as with manual logs from "cradle to grave". Working stock material that has been removed from long-term storage is tracked by use of forms that are carefully reviewed by lab members and the Responsible Official or Alternate Responsible Official. All activities for destruction and the final disposition of the agent will be documented for record keeping or, if transferred, by required Form 2 documentation which will be maintained by the Responsible Official/Alternate Responsible Official for a period of three years.

(b) (3) (A), (b) (6)

4) Plans for appropriate biosafety, biocontainment, and security of the Select Agent(s).
SR BSL-3/ABSL-3 is a modern, state-of-the-art facility, which is capable of select agent handling for CDC-designated Tier 1 BSL-3 level containment research. (b) (3) (A), (b) (6) It is a shower-out facility with two locker rooms for access/egress to and from the facility, with negative-pressure modular laboratories, double-door airlocks, and two pass-through autoclaves. The laboratory is linked to two high-efficiency incinerators on campus. The *in vitro* containment area consists of approximately (b) (3) (A), (b) of laboratory space and has three separate functional units that are made up of a large prep room and separate bacteriology and virology suites. Each of these laboratories is under negative pressure with respect to the outer corridors of the facility. Of note, duplicate equipment is available outside of the BSL-3 facility and is used for methods development, validation and training of staff under non-BSL-3 conditions. Methods can then be transferred to the BSL-3 equipment directly, making BSL-3 assay implementation more efficient. The (b) (3) (A), (b) (6) readers located in the BSL-3 are networked for data transfer. In addition, the (b) (3) (A), (b) (6) readers are network-accessible via PC anywhere to facilitate methods transfer and troubleshooting by staff. (b) (3) (A), (b) (6)

(b) (3) (A), (b) (6) Such persons are routinely advised of the potential hazards and shall comply with all entry and exit procedures as described by written procedures and training exercises conducted at SR. Protocols involving agents of an infectious or toxic nature to be used within the BSL-3 facility are reviewed and approved when deemed appropriate by the SR Institutional Biosafety Committee (IBC) before work is initiated. All personnel working in this facility are instructed on the special biological safety procedures to be used in the facility on an annual basis, and whenever there are changes in the nature of the work in the facility. Attendance to these training sessions is mandatory. The facility has a biosafety manual that describes laboratory policies and procedures for safety, first aid, decontamination, laboratory access, entrance and exiting, animal handling, biosecurity, and emergency and incident response procedures. Details of work in A/BSL-3 areas are included that explain procedures and processes while inside containment. Personnel are required to acknowledge in writing that they have read and understand all information provided in the manual.

REFERENCES

1. CDC. 2017-2018 Influenza Season Week 9 ending March 3, 2018 1600 Clifton Road Atlanta, GA 30329-4027 USA: CDC; 2018 [cited 2018 March 9, 2018]. Available from: https://www.cdc.gov/flu/weekly/index.htm.

2. CDC. FluView 2018 [cited 2018 March 6 2018.]. Available from: https://gis.cdc.gov/GRASP/Fluview/PedFluDeath.html.

3. Zanin M, Wong SS, Barman S, Kaewborisuth C, Vogel P, Rubrum A, et al. Molecular basis of mammalian transmissibility of avian H1N1 influenza viruses and their pandemic potential. Proc Natl Acad Sci U S A. 2017;114(42):11217-22. PubMed PMID: 28874549; PubMed Central PMCID: PMCPMC5651783.

4. Harfoot R, Webby RJ. H5 influenza, a global update. J Microbiol. 2017;55(3):196-203. PubMed PMID: 28243942.

5. Leung YH, To MK, Lam TS, Yau SW, Leung OS, Chuang SK. Epidemiology of human influenza A(H7N9) infection in Hong Kong. J Microbiol Immunol Infect. 2017;50(2):183-8. PubMed PMID: 26220305.

6. Kile JC, Ren R, Liu L, Greene CM, Roguski K, Iuliano AD, et al. Update: Increase in Human Infections with Novel Asian Lineage Avian Influenza A(H7N9) Viruses During the Fifth Epidemic - China, October 1, 2016-August 7, 2017. MMWR Morb Mortal Wkly Rep. 2017;66(35):928-32. PubMed PMID: 28880856; PubMed Central PMCID: PMCPMC5689040.

7. CDC. Summary of Influenza Risk Assessment Tool (IRAT) results. Atlanta, GA: US Department of Health and Human Services, CDC; 2017 [cited 2018 March 21].

8. Gubareva LV, Besselaar TG, Daniels RS, Fry A, Gregory V, Huang W, et al. Global update on the susceptibility of human influenza viruses to neuraminidase inhibitors, 2015-2016. Antiviral Res. 2017;146:12-20. PubMed PMID: 28802866; PubMed Central PMCID: PMCPMC5667636.

9. Gupta RK, Nguyen-Van-Tam JS. Oseltamivir resistance in influenza A (H5N1) infection. N Engl J Med. 2006;354(13):1423-4; author reply -4. PubMed PMID: 16571890.

10. Schaduangrat N, Phanich J, Rungrotmongkol T, Lerdsamran H, Puthavathana P, Ubol S. The significance of naturally occurring neuraminidase quasispecies of H5N1 avian influenza virus on resistance to oseltamivir: a point of concern. J Gen Virol. 2016;97(6):1311-23. PubMed PMID: 26935590; PubMed Central PMCID: PMCPMC5042091.

11. Naesens L, Stevaert A, Vanderlinden E. Antiviral therapies on the horizon for influenza. Curr Opin Pharmacol. 2016;30:106-15. PubMed PMID: 27570127.

12. Koszalka P, Tilmanis D, Hurt AC. Influenza antivirals currently in late-phase clinical trial. Influenza Other Respir Viruses. 2017;11(3):240-6. PubMed PMID: 28146320; PubMed Central PMCID: PMCPMC5410715.

13. Epstein C. Experience with favipiravir in phase 3 and implications for future studies. 5th ISIRV-AVG Conference-Prevention and Treatment of RVIs: Antivirals, Traditiional Therapies and Host-Directed Interventions; 14-16 June 2017; Singapore2017.

14. Portsmouth S, Kawaguchi K, Mastugu A, Tsuchiya K, Uehara T, editors. Cap-dependent Endonuclease Inhibitor S-033188 for the Treatment of Influenza: Results from a Phase 3, Randomized, Double-Blind, Placebo- and Active Controlled Study in Otherwise Healthy Adolescents and Adults with Seasonal Influenza. IDWeek 2017; 2017; San Diego, CA: Oxford University Press.

15. Finberg R, Lanno R, Anderson D. The Topaz trial: A phase 2B study of pimodivir (JNJ-63623872 or JNJ-872; formerly VX-787) as monotherapy or in combination with oseltamivir in the treatment of acute uncomplicated seasonal influenza. 5th ISIRV-AVG Conference-Prevention and Treatment of RVIs: Antivirals, Traditional Therapies and Host-Directed Interventions; 14-16 June 2017; Shanghai, China2017.

16. Clark MP, Ledeboer MW, Davies I, Byrn RA, Jones SM, Perola E, et al. Discovery of a novel, first-in-class, orally bioavailable azaindole inhibitor (VX-787) of influenza PB2. J Med Chem. 2014;57(15):6668-78. PubMed PMID: 25019388.

17. O'Neil B, Ison M, Hallouin-Bernard M. The Opal trial: A placebo-controlled phase 2b trial studying pimodivir plus oseltamivir vs. placebo plus oseltamivir in adult and elderly hospitalized patients with influenza A infection. 6th ESWI Conference; 10-13 September 2017; Riga, Latvia2017.

18. Te Velthuis AJ, Fodor E. Influenza virus RNA polymerase: insights into the mechanisms of viral RNA synthesis. Nat Rev Microbiol. 2016;14(8):479-93. PubMed PMID: 27396566; PubMed Central PMCID: PMCPMC4966622.

19. Zhou Z, Liu T, Zhang J, Zhan P, Liu X. Influenza A virus polymerase: an attractive target for next-generation anti-influenza therapeutics. Drug Discov Today. 2018. PubMed PMID: 29339107.

20. DuBois RM, Slavish PJ, Baughman BM, Yun MK, Bao J, Webby RJ, et al. Structural and biochemical basis for development of influenza virus inhibitors targeting the PA endonuclease. PLoS Pathog. 2012;8(8):e1002830. PubMed PMID: 22876176; PubMed Central PMCID: PMCPMC3410894.

21. Biswas SK, Nayak DP. Mutational analysis of the conserved motifs of influenza A virus polymerase basic protein 1. J Virol. 1994;68(3):1819-26. PubMed PMID: 8107244; PubMed Central PMCID: PMCPMC236644.

22. Li C, Wu A, Peng Y, Wang J, Guo Y, Chen Z, et al. Integrating computational modeling and functional assays to decipher the structure-function relationship of influenza virus PB1 protein. Sci Rep. 2014;4:7192. PubMed PMID: 25424584; PubMed Central PMCID: PMCPMC4244630.

23. Shim JM, Kim J, Tenson T, Min JY, Kainov DE. Influenza Virus Infection, Interferon Response, Viral Counter-Response, and Apoptosis. Viruses. 2017;9(8). PubMed PMID: 28805681; PubMed Central PMCID: PMCPMC5580480.

24. Nayak DP, Balogun RA, Yamada H, Zhou ZH, Barman S. Influenza virus morphogenesis and budding. Virus Res. 2009;143(2):147-61. PubMed PMID: 19481124; PubMed Central PMCID: PMCPMC2730999.

25. Urakova N, Kuznetsova V, Crossman DK, Sokratian A, Guthrie DB, Kolykhalov AA, et al. beta-D-N(4)-hydroxycytidine is a potent anti-alphavirus compound that induces high level of mutations in viral genome. J Virol. 2017. PubMed PMID: 29167335; PubMed Central PMCID: PMCPMC5774879.

26. Baranovich T, Wong SS, Armstrong J, Marjuki H, Webby RJ, Webster RG, et al. T-705 (favipiravir) induces lethal mutagenesis in influenza A H1N1 viruses in vitro. J Virol. 2013;87(7):3741-51. PubMed PMID: 23325689; PubMed Central PMCID: PMCPMC3624194.

27. Bank C, Renzette N, Liu P, Matuszewski S, Shim H, Foll M, et al. An experimental evaluation of drug-induced mutational meltdown as an antiviral treatment strategy. Evolution. 2016;70(11):2470-84. PubMed PMID: 27566611.

28. Tran V, Moser LA, Poole DS, Mehle A. Highly sensitive real-time in vivo imaging of an influenza reporter virus reveals dynamics of replication and spread. J Virol. 2013;87(24):13321-9. PubMed PMID: 24089552; PubMed Central PMCID: PMCPMC3838222.

29. Hastings JC, Selnick H, Wolanski B, Tomassini JE. Anti-influenza virus activities of 4-substituted 2,4-dioxobutanoic acid inhibitors. Antimicrob Agents Chemother. 1996;40(5):1304-7. PubMed PMID: 8723491; PubMed Central PMCID: PMCPMC163316.

30. Gu W, Gallagher GR, Dai W, Liu P, Li R, Trombly MI, et al. Influenza A virus preferentially snatches noncoding RNA caps. RNA. 2015;21(12):2067-75. PubMed PMID: 26428694; PubMed Central PMCID: PMCPMC4647461.

31. Furuta Y, Gowen BB, Takahashi K, Shiraki K, Smee DF, Barnard DL. Favipiravir (T-705), a novel viral RNA polymerase inhibitor. Antiviral Res. 2013;100(2):446-54. PubMed PMID: 24084488; PubMed Central PMCID: PMC3880838.

32. Baell JB, Holloway GA. New substructure filters for removal of pan assay interference compounds (PAINS) from screening libraries and for their exclusion in bioassays. J Med Chem. 2010;53(7):2719-40. PubMed PMID: 20131845.

33. Simeonov A, Jadhav A, Thomas CJ, Wang Y, Huang R, Southall NT, et al. Fluorescence spectroscopic profiling of compound libraries. J Med Chem. 2008;51(8):2363-71. PubMed PMID: 18363325.

34. Smee DF, Tarbet EB, Furuta Y, Morrey JD, Barnard DL. Synergistic combinations of favipiravir and oseltamivir against wild-type pandemic and oseltamivir-resistant influenza A virus infections in mice. Future Virol. 2013;8(11):1085-94. PubMed PMID: 24563658; PubMed Central PMCID: PMCPMC3929310.

35. Basu D, Walkiewicz MP, Frieman M, Baric RS, Auble DT, Engel DA. Novel influenza virus NS1 antagonists block replication and restore innate immune function. J Virol. 2009;83(4):1881-91. PubMed PMID: 19052087; PubMed Central PMCID: PMCPMC2643796.

36. Menachery VD, Eisfeld AJ, Schafer A, Josset L, Sims AC, Proll S, et al. Pathogenic influenza viruses and coronaviruses utilize similar and contrasting approaches to control interferon-stimulated gene responses. mBio. 2014;5(3):e01174-14. PubMed PMID: 24846384; PubMed Central PMCID: PMCPMC4030454.

37. Menachery VD, Schafer A, Burnum-Johnson KE, Mitchell HD, Eisfeld AJ, Walters KB, et al. MERS-CoV and H5N1 influenza virus antagonize antigen presentation by altering the epigenetic landscape. Proc Natl Acad Sci U S A. 2018;115(5):E1012-E21. PubMed PMID: 29339515.

38. Cody R, Stewart D, DeForni M, Moore M, Dallaire B, Azarnia N, et al. Multicenter phase II study of brequinar sodium in patients with advanced breast cancer. Am J Clin Oncol. 1993;16(6):526-8. PubMed PMID: 8256771.

39. Vyas VK, Ghate M. Recent developments in the medicinal chemistry and therapeutic potential of dihydroorotate dehydrogenase (DHODH) inhibitors. Mini Rev Med Chem. 2011;11(12):1039-55. PubMed PMID: 21861807.

40. Das P, Deng X, Zhang L, Roth MG, Fontoura BM, Phillips MA, et al. SAR Based Optimization of a 4-Quinoline Carboxylic Acid Analog with Potent Anti-Viral Activity. ACS Med Chem Lett. 2013;4(6):517-21. PubMed PMID: 23930152; PubMed Central PMCID: PMCPMC3733392.

41. Aronov AM. Predictive in silico modeling for hERG channel blockers. Drug Discov Today. 2005;10(2):149-55. PubMed PMID: 15718164.

42. Finlayson K, Witchel HJ, McCulloch J, Sharkey J. Acquired QT interval prolongation and HERG: implications for drug discovery and development. Eur J Pharmacol. 2004;500(1-3):129-42. PubMed PMID: 15464027.

43. Hoffmann E, Krauss S, Perez D, Webby R, Webster RG. Eight-plasmid system for rapid generation of influenza virus vaccines. Vaccine. 2002;20(25-26):3165-70. PubMed PMID: 12163268.

44. Resa-Infante P, Recuero-Checa MA, Zamarreno N, Llorca O, Ortin J. Structural and functional characterization of an influenza virus RNA polymerase-genomic RNA complex. J Virol. 2010;84(20):10477-87. PubMed PMID: 20702645; PubMed Central PMCID: PMCPMC2950564.

45. Dias A, Bouvier D, Crepin T, McCarthy AA, Hart DJ, Baudin F, et al. The cap-snatching endonuclease of influenza virus polymerase resides in the PA subunit. Nature. 2009;458(7240):914-8. PubMed PMID: 19194459.

46. Datta K, Wolkerstorfer A, Szolar OH, Cusack S, Klumpp K. Characterization of PA-N terminal domain of Influenza A polymerase reveals sequence specific RNA cleavage. Nucleic Acids Res. 2013;41(17):8289-99. PubMed PMID: 23847103; PubMed Central PMCID: PMCPMC3783182.

47. Yuan P, Bartlam M, Lou Z, Chen S, Zhou J, He X, et al. Crystal structure of an avian influenza polymerase PA(N) reveals an endonuclease active site. Nature. 2009;458(7240):909-13. PubMed PMID: 19194458.

48. Swale C, Monod A, Tengo L, Labaronne A, Garzoni F, Bourhis JM, et al. Structural characterization of recombinant IAV polymerase reveals a stable complex between viral PA-PB1 heterodimer and host RanBP5. Sci Rep. 2016;6:24727. PubMed PMID: 27095520; PubMed Central PMCID: PMCPMC4837377.

49. Neumann G, Watanabe T, Ito H, Watanabe S, Goto H, Gao P, et al. Generation of influenza A viruses entirely from cloned cDNAs. Proc Natl Acad Sci U S A. 1999;96(16):9345-50. PubMed PMID: 10430945; PubMed Central PMCID: PMCPMC17785.

50. Hoffmann E, Stech J, Guan Y, Webster RG, Perez DR. Universal primer set for the full-length amplification of all influenza A viruses. Arch Virol. 2001;146(12):2275-89. PubMed PMID: 11811679.

51. Nguyen JT, Hoopes JD, Le MH, Smee DF, Patick AK, Faix DJ, et al. Triple combination of amantadine, ribavirin, and oseltamivir is highly active and synergistic against drug resistant influenza virus strains in vitro. PLoS One. 2010;5(2):e9332. PubMed PMID: 20179772; PubMed Central PMCID: PMC2825274.

52. Smee DF, Prichard MN. Comparison of three dimensional synergistic analyses of percentage versus logarithmic data in antiviral studies. Antiviral Res. 2017;145:1-5. PubMed PMID: 28676302; PubMed Central PMCID: PMCPMC5575975.

53. Zarogiannis SG, Noah JW, Jurkuvenaite A, Steele C, Matalon S, Noah DL. Comparison of ribavirin and oseltamivir in reducing mortality and lung injury in mice infected with mouse adapted A/California/04/2009

(H1N1). Life Sci. 2012;90(11-12):440-5. PubMed PMID: 22269828; PubMed Central PMCID: PMCPMC4934888.

54. Oh DY, Hurt AC. Using the Ferret as an Animal Model for Investigating Influenza Antiviral Effectiveness. Front Microbiol. 2016;7:80. PubMed PMID: 26870031; PubMed Central PMCID: PMCPMC4740393.

RESOURCE AND DATA SHARING

Sharing of data generated by Project 4 is an essential part of our proposed activities and will be carried out in several different ways. We will make our results available both to the community of scientists interested in antiviral therapy and viral biology to avoid unintentional duplication of research.

Our plan includes the following:

Presentations at national scientific meetings. We anticipate two presentations at international scientific meetings. Dr. Prichard frequently presents data at the International Conference on Antiviral Research, and data could be presented as either and oral presentation or a poster presentation. This is an important annual meeting in the field and the he was elected to its Board of Directors and served as the Program Chair for 5 years. He is an active participant of this focused group.

Publications. The most important means of sharing data is through the publication of data in peer reviewed scientific journals. Dr. Prichard is committed to the dissemination of results from his laboratory by this mechanism and has published more than 110 manuscripts, book chapters, or reviews in peer reviewed journals. This is particularly important in the field of antiviral research such that the antiviral activity of the same compounds are not tested by several laboratories.

Data documentation. All research data collected during the grant period will be documented in various forms, including in laboratory notebooks and electronically. Compound structures, screening data, and assay performance data will be archived electronically in .txt file format and analyzed electronically using (b) (3) (A), (b) (4) . Regardless of the documentation method, the data will be documented such that reconstruction and reproduction of experiments, accurate interpretation, and future analyses can be undertaken.

Researchers' efforts. We anticipate that we will generate some data that may be of use to certain sectors of the governmental and/or research community prior to widespread disclosure. Southern Research and the University of Alabama at Birmingham (UAB) have a consistent history of sharing such data, as do the Core Leaders and Key Personnel from the other institutions participating in this U19 program proposal. The Core Leader and Co-Core Leader have freely shared data with NIAID in the past when involved with assay development and HTS screening for influenza antivirals, and will initiate frequent communications with the program manager and other researchers. We will continue to share data under the support of the individual investigator institutions, invoking such conditions as confidentiality, establishing collaborative relationships, or other such conditions that protect the rights of proprietary data, as detailed below in protection of Intellectual Property Rights.

Public disclosure. The sharing of final research data will occur by oral disclosure and written scientific publications after appropriate patent protection is in place. These methods are effective means of sharing data which also provide an opportunity for peer-review.

- Oral disclosure. The scientists engaged in the research described in this proposal actively participate in scientific meetings, where current data are freely shared.
- Publication. We expect that final research data will be shared in peer-reviewed scientific journals. Such journals are widely read by scientists engaged in antiviral and biodefense research as well as in other fields. Therefore, we anticipate that publication in journals will permit broad sharing of research data.

Protection of Intellectual Property Rights. Consistent with the policies at the participating institutions of this proposal and NIH policy on intellectual property rights, the laboratories of the key personnel will make all materials and reagents produced under this Project available for use at other academic or not-for-profit institutions at no cost, except for standard maintenance and transportation expenses. Southern Research, UAB, and other collaborating institutions will reserve the right to use these reagents for education, research, or other not-for-profit purposes. These institutions may establish non-exclusive commercial licenses granting their rights to use the materials at specific for-profit entities; in these cases, the institutions will maintain the right to grant non-exclusive licenses for the use of these materials by academic or not-for-profit institutions. Requests for materials from for-profit corporations to use them commercially may be negotiated by the Intellectual Property Group of the participating institutions in collaboration with the other institutions as necessary.

AUTHENTICATION OF KEY BIOLOGICAL AND CHEMICAL RESOURCES

The proposed Research Program for Project 4 incorporates and relies on a number of biological and chemical resources through a broad range of approaches at a frequency that is appropriate for each line of inquiry. The methods for ensuring identity and validity of these resources are as follows:

Chemicals: Chemicals commercially acquired are authenticated by the external vendors and additional tests are conducted as deemed necessary. New compounds will be synthesized by the Medicinal Chemistry and Lead Development Core at Southern Research. Each new compound will be evaluated for structural integrity (1H NMR, LCMS) and purity (HPLC) prior to biological evaluation. Additional characterization of material will be performed on scale-up GLP material synthesis for GLP tox studies and animal studies.

Plasmids: Plasmids acquired from external sources or constructed in-house are always validated by restriction analysis and DNA sequencing. Plasmid clones referred to in the application were obtained from the laboratory (b) (3) (A), (b) (6) and plasmids pHW191, 192, 193, 194, 195, 196, 197, and 198. New stocks of the DNA were prepared and sequenced to confirm the integrity of the influenza genes by (b) (3) (A), (b) (6)

Antibodies: All antibodies are purchased as authenticated materials directly from commercial vendors. The specificity of antibodies are validated through Western blotting analysis using antibodies-specific antigens. The antibody referred to in the application is Influenza A M2 mouse mAb IgG (b) (3) (A), (b) (6)

Cell Lines: Cell lines are acquired from ATTC and other commercial cell line providers, who authenticate their lots by a variety of specific tests (e.g. by assessing Short Tandem Repeats in the DNA of each cell line). This information is readily available on the vendor's web site. Cell lines are tested regularly for mycoplasma infection with available kits based on specific PCR.

For the information relevant to this application, key cell lines used were all purchased from the (b) (3) (A), (b) (6) . Cells used include the following:

MDCK (NBL-2) (ATCC® CCL-34™)

A549 (ATCC® CCL-185™)

293 [HEK-293] (ATCC® CRL-1573™)

All cell lines were obtained with a certificate of analysis from the vendor indicating that the cells were authenticated by isoenzyme, DNA profile, and cytogenetic analysis. STR profiling is also available from the company.

The Master Cell Bank for cell lines was tested to authenticate the critical reagents. Each cell line is used within a prescribed number of passages before a new aliquot is thawed from the Master Cell Bank to minimize genetic drift.

Mycoplasma testing was also performed on each Master Cell Bank, using the MycoAlert Mycoplasma Detection kit.

Viruses:

(b) (3) (A), (b) (6)

Vertebrate Animals: Animals are purchased from reputable vendors. In particular:

- Mice – (b) (3) (A), (b) (6)
- Ferrets - (b) (3) (A), (b) (6)

Equipment and measurement instruments: Regular maintenance is performed on all equipment within the Infectious Diseases Department, not only for efficient work flow, but also for reproducible results on tests using biological and/or chemical resources. Measurement equipment and devices such as pipettes, balances, pH-meters, cell counters, etc. are regularly calibrated. In addition, standards or controls are used or validated on regular intervals.

Results: To authenticate findings, it is a standard of our labs to include positive and negative controls in all studies, to run multiple tests for each assay, and to perform numerous complementary assays.

Data Reporting: Publication of research results in peer-reviewed journals is expected of all members of the Project Team, providing an additional level of authentication. Facilitating this, all personnel have access to training and assistance offered by the (b) (3) (A), (b) (6), in subjects related to publication and data reporting. In addition, we will maintain a strong presence at multiple regional, national, and international conferences, including the NIAID CETR Program Meeting.

Statistical analyses and data rigor/reproducibility. Group sizes for PK studies in animal models will enable, at a minimum, calculation of mean and standard deviation values for data parameters, while the number of dose groups will be consistent with the recommendations of the FDA, the OECD and the International Conference on Harmonization of Technical Requirements of Pharmaceuticals for Human Use (ICH).
In vivo efficacy studies will include 10 mice or 6 ferrets/time point based on our statistical power calculations (see Vertebrate Animal section). This number of animals will be sufficient to detect differences between groups with at least an 80% power and 0.05 significance. Statistical analyses will include unpaired *t*-tests or non-parametric tests when comparing two independent groups (i.e. placebo and drug treated) and ANOVA tests when comparing multiple independent groups (i.e. multiple drug comparisons to placebo).

Consideration of relevant biological variables All experiments in animals will include an equal distribution of males and females and will be conducted in sexually mature adult animals. Animals will be maintained in temperature- and humidity- controlled space within the animal facilities. Protocols to test the efficacy of different drugs are designed in a way that animals are assigned randomly to each treatment group. When possible, treatments will be assigned blindly to the experimenter by another individual in the lab. Finally, for cell culture experiments, biological replicates of different passages on different days will be performed so that we can minimize any pseudo-replication that is inherent with cell culture experiments.

Notice of Award

RESEARCH PROJECT COOPERATIVE AGREEMENT **Federal Award Date:** 02/27/2020

Department of Health and Human Services
National Institutes of Health

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Grant Number: 5U19AI142759-02
FAIN: U19AI142759

Principal Investigator(s):
RICHARD J. WHITLEY, MD

Project Title: Antiviral Drug Discovery and Development Center (AD3C)

(b) (3) (A), (b) (6)
UNIVERSITY OF ALABAMA AT BIRMINGHAM
1720 2nd Avenue South, (b) (3)
Birmingham, AL 352940111 (A) (b) (6)

Award e-mailed to: OSP-NGA@mail.ad.uab.edu

Period Of Performance:
Budget Period: 03/01/2020 – 02/28/2021
Project Period: 03/07/2019 – 02/29/2024

Dear Business Official:

The National Institutes of Health hereby awards a grant in the amount of $7,500,000 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to UNIVERSITY OF ALABAMA AT BIRMINGHAM in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 31 USC 6305 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as "Research reported in this publication was supported by the National Institute Of Allergy And Infectious Diseases of the National Institutes of Health under Award Number U19AI142759. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health." Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination.

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator's Financial Conflict of Interest (FCOI), in accordance with the 2011 revised regulation at 42 CFR Part 50 Subpart F. The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module. The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards. Consult the NIH website http://grants.nih.gov/grants/policy/coi/ for a link to the regulation and additional important information.

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Sincerely yours,

Regina E. Kitsoulis
Grants Management Officer
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Additional information follows

NIH NGA R | Version: 56 - 12/26/2018 2:22:00 PM| Generated on: 2/27/2020 12:02:43 AM

SECTION I – AWARD DATA – 5U19AI142759-02

Award Calculation (U.S. Dollars)

Salaries and Wages	$151,843
Fringe Benefits	$45,498
Personnel Costs (Subtotal)	$197,341
Consultant Services	$18,500
Materials & Supplies	$24,659
Travel	$20,288
Other	$17,730
Subawards/Consortium/Contractual Costs	$7,082,688
Publication Costs	$2,500
Federal Direct Costs	$7,363,706
Federal F&A Costs	$136,294
Approved Budget	$7,500,000
Total Amount of Federal Funds Obligated (Federal Share)	$7,500,000
TOTAL FEDERAL AWARD AMOUNT	$7,500,000
AMOUNT OF THIS ACTION (FEDERAL SHARE)	$7,500,000

SUMMARY TOTALS FOR ALL YEARS		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
2	$7,500,000	$7,500,000
3	$7,500,000	$7,500,000
4	$7,500,000	$7,500,000
5	$7,500,000	$7,500,000

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

Fiscal Information:

CFDA Name:	Allergy and Infectious Diseases Research
CFDA Number:	93.855
EIN:	1636005396A6
Document Number:	UAI142759A
PMS Account Type:	P (Subaccount)
Fiscal Year:	2020

IC	CAN	2020	2021	2022	2023
AI	8472315	$7,500,000	$7,500,000	$7,500,000	$7,500,000

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

NIH Administrative Data:
PCC: M65B B / **OC**: 41029 / **Released**: (b) (6) 02/26/2020
Award Processed: 02/27/2020 12:02:43 AM

SECTION II – PAYMENT/HOTLINE INFORMATION – 5U19AI142759-02

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III – TERMS AND CONDITIONS – 5U19AI142759-02

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

a. The grant program legislation and program regulation cited in this Notice of Award.
b. Conditions on activities and expenditure of funds in other statutory requirements, such as

NIH NGA 8.1 Version: 88 12/20/2019 2:32:00 PM Generated on: 2/27/2020 12:02:43 AM

those included in appropriations acts.
c. 45 CFR Part 75.
d. National Policy Requirements and all other requirements described in the NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
e. Federal Award Performance Goals: As required by the periodic report in the RPPR or in the final progress report when applicable.
f. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm for certain references cited above.)

Research and Development (R&D): All awards issued by the National Institutes of Health (NIH) meet the definition of "Research and Development" at 45 CFR Part§ 75.2. As such, auditees should identify NIH awards as part of the R&D cluster on the Schedule of Expenditures of Federal Awards (SEFA). The auditor should test NIH awards for compliance as instructed in Part V, Clusters of Programs. NIH recognizes that some awards may have another classification for purposes of indirect costs. The auditor is not required to report the disconnect (i.e., the award is classified as R&D for Federal Audit Requirement purposes but non-research for indirect cost rate purposes), unless the auditee is charging indirect costs at a rate other than the rate(s) specified in the award document(s).

This institution is a signatory to the Federal Demonstration Partnership (FDP) Phase VI Agreement which requires active institutional participation in new or ongoing FDP demonstrations and pilots.

Carry over of an unobligated balance into the next budget period requires Grants Management Officer prior approval.

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the System for Award Management (SAM). Should a consortium/subaward be issued under this award, a DUNS requirement must be included. See http://grants.nih.gov/grants/policy/awardconditions.htm for the full NIH award term implementing this requirement and other additional information.

This award has been assigned the Federal Award Identification Number (FAIN) U19AI142759. Recipients must document the assigned FAIN on each consortium/subaward issued under this award.

Based on the project period start date of this project, this award is likely subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170. There are conditions that may exclude this award; see http://grants.nih.gov/grants/policy/awardconditions.htm for additional award applicability information.

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: http://publicaccess.nih.gov/.

In accordance with the regulatory requirements provided at 45 CFR 75.113 and Appendix XII to 45 CFR Part 75, recipients that have currently active Federal grants, cooperative agreements, and procurement contracts with cumulative total value greater than $10,000,000 must report and maintain information in the System for Award Management (SAM) about civil, criminal, and administrative proceedings in connection with the award or performance of a Federal award that reached final disposition within the most recent five-year period. The recipient must also make semiannual disclosures regarding such proceedings. Proceedings information will be made publicly available in the designated integrity and performance system (currently the Federal

Awardee Performance and Integrity Information System (FAPIIS)). Full reporting requirements and procedures are found in Appendix XII to 45 CFR Part 75. This term does not apply to NIH fellowships.
Treatment of Program Income:
Additional Costs

SECTION IV – AI Special Terms and Conditions – 5U19AI142759-02

Clinical Trial Indicator: No
This award does not support any NIH-defined Clinical Trials. See the NIH Grants Policy Statement Section 1.2 for NIH definition of Clinical Trial.

This award is issued as a Cooperative Agreement, a financial assistance mechanism in which substantial NIH scientific and/or programmatic involvement is anticipated in the performance of the activity. This award is subject to the Terms and Conditions of Award as set forth in Section VI: Award Administrative Information of **RFA AI-17-042**, "**Centers of Excellence for Translational Research (CETR) (U19 Clinical Trial Not Allowed)**," posted date **11/30/2017**, which are hereby incorporated by reference as special terms and conditions of this award.

This RFA may be accessed at: http://grants.nih.gov/grants/guide/index.html

---
This Notice of Award (NoA) includes funds for activity with **Southern Research Institute** in the amount of **$3,795,723** (**$1,747,626** direct costs + **$2,048,097** F&A costs).

This Notice of Award (NoA) includes funds for activity with **Emory University** in the amount of **$321,300** (**$180,000** direct costs + **$141,300** F&A costs).

This Notice of Award (NoA) includes funds for activity with **Vanderbilt University** in the amount of **$458,770** (**$290,361** direct costs + **$168,409** F&A costs).

This Notice of Award (NoA) includes funds for activity with **University of North Carolina Chapel Hill** in the amount of **$823,733** (**$532,034** direct costs + **$291,699** F&A costs).

This Notice of Award (NoA) includes funds for activity with **Oregon Health and Science University** in the amount of **$759,924** (**$493,457** direct costs + **$266,467** F&A costs).

This Notice of Award (NoA) includes funds for activity with **University of Colorado at Denver** in the amount of **$320,119** (**$160,059** direct costs + **$160,060** F&A costs).

This Notice of Award (NoA) includes funds for activity with **University of Texas Medical Branch** in the amount of **$320,966** (**$203,143** direct costs + **$117,823** F&A costs).

This Notice of Award (NoA) includes funds for activity with **Washington University** in the amount of **$353,383** (**$231,720** direct costs + **$121,663** F&A costs).

In addition to the PI, any absence, replacement, or substantial reduction in effort of the following individual(s) below, requires the written prior approval of the National Institutes of Health awarding component.

(b) (3) (A), (b) (6)

Awardees who conduct research involving Select Agents (see 42 CFR 73 for the Select Agent list; and 7 CFR 331 and 9 CFR 121 for the relevant animal and plant pathogens at http://www.selectagents.gov/Regulations.html) must complete registration with CDC (or APHIS, depending on the agent) before using NIH funds. No funds can be used for research involving Select Agents if the final registration certificate is denied.

Prior to conducting a restricted experiment with a Select Agent or Toxin, awardees must notify the NIAID and must request and receive approval from CDC or APHIS.

NIH NGA R | Version: 58 - 1/23/2017 2:32:00 PM | Generated on: 2/27/2020 12:02:20 AM

Select Agents:
Awardee of a project that at any time involves a restricted experiment with a select agent, is responsible for notifying and receiving prior approval from the NIAID. Please be advised that changes in the use of a Select Agent will be considered a change in scope and require NIH awarding office prior approval. The approval is necessary for new select agent experiments as well as changes in on-going experiments that would require change in the biosafety plan and/or biosafety containment level. An approval to conduct a restricted experiment granted to an individual cannot be assumed an approval to other individuals who conduct the same restricted experiment as defined in the Select Agents Regulation 42 CFR Part 73, Section 13.b (http://www.selectagents.gov/Regulations.html).

Highly Pathogenic Agent:
NIAID defines a Highly Pathogenic Agent as an infectious Agent or Toxin that may warrant a biocontainment safety level of BSL3 or higher according to the current edition of the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL) (http://www.cdc.gov/OD/ohs/biosfty/bmbl5/bmbl5toc.htm). Research funded under this grant must adhere to the BMBL, including using the BMBL-recommended biocontainment level at a minimum. If your Institutional Biosafety Committee (or equivalent body) or designated institutional biosafety official recommend a higher biocontainment level, the highest recommended containment level must be used.
When submitting future Progress Reports indicate at the beginning of the report:

If no research with a Highly Pathogenic Agent or Select Agent has been performed or is planned to be performed under this grant.

If your IBC or equivalent body or official has determined, for example, by conducting a risk assessment, that the work being planned or performed under this grant may be conducted at a biocontainment safety level that is lower than BSL3.

If the work involves Select Agents and/or Highly Pathogenic Agents, also address the following points:

Any changes in the use of the Agent(s) or Toxin(s) including its restricted experiments that have resulted in a change in the required biocontainment level, and any resultant change in location, if applicable, as determined by your IBC or equivalent body or official.

If work with a new or additional Agent(s)/Toxin(s) is proposed in the upcoming project period, provide:

- A list of the new and/or additional Agent(s) that will be studied;
- A description of the work that will be done with the Agent(s), and whether or not the work is a restricted experiment;
- The title and location for each biocontainment resource/facility, including the name of the organization that operates the facility, and the biocontainment level at which the work will be conducted, with documentation of approval by your IBC or equivalent body or official. It is important to note if the work is being done in a new location.

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These individuals work together in overall project administration. Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

Grants Management Specialist: Elizabeth R. Sihombing
Email: elizabeth.sihombing@nih.gov **Phone**: 240-669-5530

Program Official: Maureen J. Beanan
Email: beananm@mail.nih.gov **Phone**: 240-292-0999

NIH NGA R | Version: 5S - 12/28/2016 2:32:00 PM | Generated on: Jul 7 2020 12:07:57 AM

SPREADSHEET SUMMARY
GRANT NUMBER: 5U19AI142759-02

INSTITUTION: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Budget	Year 2	Year 3	Year 4	Year 5
Salaries and Wages	$151,843	$147,124	$147,549	$146,934
Fringe Benefits	$45,498	$46,871	$47,006	$46,810
Personnel Costs (Subtotal)	$197,341	$193,995	$194,555	$193,744
Consultant Services	$18,500	$17,714	$17,765	$17,691
Materials & Supplies	$24,659	$14,129	$14,169	$14,111
Travel	$20,288	$26,993	$27,070	$26,957
Other	$17,730	$18,136	$18,188	$18,113
Subawards/Consortium/Contractual Costs	$7,082,688	$7,092,604	$7,091,430	$7,093,131
Publication Costs	$2,500	$3,374	$3,384	$3,370
TOTAL FEDERAL DC	$7,363,706	$7,366,945	$7,366,561	$7,367,117
TOTAL FEDERAL F&A	$136,294	$133,055	$133,439	$132,883
TOTAL COST	$7,500,000	$7,500,000	$7,500,000	$7,500,000

Facilities and Administrative Costs	Year 2	Year 3	Year 4	Year 5
F&A Cost Rate 1	48.5%	48.5%	48.5%	48.5%
F&A Cost Base 1	$281,018	$274,341	$275,131	$273,986
F&A Costs 1	$136,294	$133,055	$133,439	$132,883

A. OVERALL COVER PAGE

Project Title: Antiviral Drug Discovery and Development Center (AD3C)	
Grant Number: 5U19AI142759-02	**Project/Grant Period:** 03/07/2019 - 02/29/2024
Reporting Period: 03/07/2019 - 02/29/2020	**Requested Budget Period:** 03/01/2020 - 02/28/2021
Report Term Frequency: Annual	**Date Submitted:** 12/20/2019
Program Director/Principal Investigator Information: RICHARD J WHITLEY , AB MD **Phone number:** 205-934-5316 **Email:** rwhitley@peds.uab.edu	**Recipient Organization:** UNIVERSITY OF ALABAMA AT BIRMINGHAM UNIVERSITY OF ALABAMA AT BIRMINGHAM (b) (3) (A), (b) (6) **DUNS:** 063690705 **EIN:** 1636005396A6 **RECIPIENT ID:**
Change of Contact PD/PI: N/A	
Administrative Official: (b) (3) (A), (b) (6) 701 20th Street South (b) (3) Birmingham, AL 35294 (A) (b) **Phone number:** (b) (3) (A), (b) **Email:** (b) (3) (A), (b)	**Signing Official:** (b) (3) (A), (b) (6) 701 20th Street South (b) (3) Birmingham, AL 35294 (A) (b) **Phone number:** (b) (3) (A), (b) **Email:** (b) (3) (A), (b)
Human Subjects: No	**Vertebrate Animals:** Yes
hESC: No	**Inventions/Patents:** Yes If yes, previously reported: Yes

B. OVERALL ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

The overarching goal of this application is to deliver novel small molecule therapeutics for emerging and reemerging viral infections that work by targeting mechanisms essential to viral replication. Through the CETR program, the existing Projects and Cores of the Antiviral Drug Development and Discovery Center (AD3C) have already identified lead molecules suitable for proof-of-principle testing in animal models of infection, which will facilitate medicinal chemistry efforts to optimize potency, selectivity, and pharmacokinetics, including bioavailability. We will utilize the existing data and compounds going forward, but also will evaluate a limited number of additional, novel compounds provided by our collaborators, the Emory Institute for Drug Development (EIDD) and Gilead Sciences. We have established four Projects – each of which addresses infections identified as high priority by the National Institute of Allergy and Infectious Diseases (NIAID). Members of several genera of RNA viruses will be studied in this proposal, as they are major causes of human disease, bioterrorist threats, or emerging infectious diseases, and pharmacological control of these viruses remains limited. Our Projects will focus on (1) coronaviruses that cause Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS), (2) alphaviruses causing Venezuelan equine encephalitis virus (VEEV) and chikungunya, 3) flaviviruses (dengue, West Nile virus, and Zika) and 4) influenza A virus. The Projects will be supported by three Cores: the Administrative Core (Core A), the Assay Core (Core B) and the Medicinal Chemistry and Lead Development Core (MCLDC; Core C). The organization and interaction between all Projects and Cores will be monitored by the Administrative Core. An Executive Committee (EC) will consist of all Project and Core leaders to review data on monthly conference calls, in order to provide further direction and foster project interactions. Finally, an external Scientific Advisory Committee (SAC) will be established to provide evaluation of the project progress and facilitate "Go/No-Go" decisions on a regular basis.

This CETR program integrates the work of nine premier laboratories with proven records of collaboration and expertise in viral pathogenesis, host immunity, mechanisms of antiviral gene function, drug screening and modification, and chemical biology. The common theme of this application is the identification and development of compounds that target viral replication and infection. The experimental strategies in each of the four Projects include a comprehensive analysis of the mechanism of action of identified lead molecules.

There is slight variation in the Specific Aims of each Project, but overall, the application is structured such that the Aims are parallel with one another. This has been done intentionally to further synergize the interaction of the Projects and maximize outcomes. Our overall Specific Aims are:

AIM 1 – Utilize hit and lead compounds to drive platform expansion by the MCLDC. We will accelerate this process by performing a focused evaluation of nucleosides and nucleotides from the EIDD, which is integrated in this Core, and (b) (3) (A), (b) (6) in order to optimize potency and drug-like properties.
We hypothesize that blending existing leads along with those newly identified will accelerate the drug discovery process.

AIM 2 – Advance compounds to profiling, mechanism of action, and resistance studies, utilizing the pharmaceutical experience of investigators in this program as well as the SAC. Further, 'actives' will be cross-screened against other viral targets in the program.
We hypothesize that select leads can be identified with unique profiles of activity and resistance, allowing these compounds to move rapidly to proof-of-principle studies in vivo in Aim 3.

AIM 3 – Advance lead molecules to animal testing beginning with formulation, pharmacokinetics, biodistribution, and therapeutic proof-of-principle animal model evaluation. In later years, combinations of molecules will be tested to evaluate for synergy and minimize the emergence of resistance.
We hypothesize that proof-of-principle animal testing can be initiated with existing leads and formulations and will result in a candidate molecule for advancement into human studies, following preclinical toxicology assessments.

Although the aims are similar in the Projects, the compound status in each will vary due to a natural evolution of compound progression in the previous grant period. Nonetheless, all Projects should deliver lead molecules that have an acceptable therapeutic index as defined by efficacy in in vitro and in vivo animal models. In fact, we expect that we will evaluate these lead molecules in the first two years of the grant period, followed by initiation of preclinical IND-enabling studies.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: 191212_SACnotesAD3C_Final.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

For this reporting period, is there one or more Revision/Supplement associated with this award for which reporting is required?

No

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

File uploaded: Overall B.4 Y1.pdf

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

One major overall goal for year 2 is to continue to support collaboration among all projects and cores. Project leaders continue to interact and are highly motivated to promote progress among teams.

Incorporating modeling studies for protein targets was identified by the scientific advisory committee and will be prioritized.

Additional project specific and core goals are reported in the respective components.

AD3C External Scientific Advisory Committee
2019 Annual Report

The Annual AD3C investigators meeting was held on November 13-14, 2019 in Birmingham, AL. Four of the five SAC committee members participated either in person (Kara Carter) or via Turbo Meeting webinar/teleconference (Rick Keenan, Tom Shenk and Mike Bray). Following presentations by the four AD3C project investigators, a preliminary project review was completed by external SAC members. Stephanie Moore and Mary Wyatt Bowers were present to lead discussion and record notes.

Overall program review:
Strengths: As a whole, Kara Carter, who served on the external advisory board for the previous grant, noted that all projects had made good progress in this past year. Each seems to have a profile definition of the molecules which had not been achieved in past reviews. All agreed that integrating the cores' presentation into each project presentation was an effective means of highlighting the accomplishments of both. Cores are definitely highly functional and strong. The SAC was very impressed with the collaboration among all projects and cores as well as the caliber of the projects. It was noted that the academic/industry partnership that has been developed should serve as a model for other drug discovery programs.

Weaknesses: As noted in individual projects below, a standard thermal shift assay should be used in all projects to either identify or confirm the molecular target of compounds, but it doesn't appear that anyone is using it now. Also, all teams should include a structural biologist who can collaborate with a computational chemist in developing research plans.

Future Directions:

- (b) (3) (A), (b) (6) suggested the creation of a 'mega-table' with compound information (ADME) across projects regarding cross-reactivity across viruses with compounds and general screening. This idea was reiterated by the SAC.
- It was also suggested that those in academic groups might benefit from a tutorial on data analysis for assays related to drug development and discovery.

Individual Project Reviews:
Project 1 – Coronaviruses
Strengths: The project, in particular, now has a definition of profile for lead.
Assays are nice and the inclusion of human airway epithelial cells is an important system to keep using.

Weaknesses: The largest issue is the lack of chemical value. There does not appear to be a well-defined future direction for hits, and concerns were expressed about attempting to move compounds forward without relaxing the criteria too much. There was concern that there were few hits to investigate, other than possible remdesivir and β-d-N^4-hydroxycytidine (NHC)

analogs. The *in vivo* studies with NHC required a very high dose (500 mg/kg) to completely block SARS-CoV replication, which is likely not a reasonable path forward.

Future directions:

- As noted above, this project could benefit from analoging remdesivir or NHC – compounds known to have some efficacy against coronaviruses- and work from there.
- Specifically, since the NHC data was intriguing, it was highly recommended that MOA studies be started for RNA polymerase using thermal shift (CETSA). It was suggested that SR might have some resources that could benefit this area – such as their Chemistry Department's Structural Biology Laboratory with (b) (3) (A), (b) Even if outsourcing is required, the SAC suggested that modeling studies could help to identify better chemical value. Another resource suggested was Project 3's group leader, Pei-Yong Shi; his expertise might be helpful here.
- Project 1 should continue the use of primary cells compared to transformed cell lines as these studies are very important factor for translational application.

Project 2 – Alphaviruses

Strengths: The SAC felt this project was the front runner and was the most advanced project of the four. Members were glad to know that compounds are being considered leads even without activity against VEEV; however, they did note that if there are compounds that have anti-VEEV activity, they should be made a priority. Chemistry is very advanced for this project. It was noted that this project is in very good shape, but should only focus on the most promising compounds with the best overall properties.

Weaknesses: The general concentration in the viral titer reduction assays was 10 micromolar and there is concern that apparent antiviral activity could indicate toxicity and therefore may be an artifact and not actual activity. Also, there was not a clear indication of what the next steps were going to be as the project moves forward (see suggestions below).

Future directions:

- Given its activity in a mouse model of CHIKV infection, compound 42718 deserves top priority. The compound 33001 was thought to be likely a host target and the group liked the suggestion of using biotinylated compounds to identify the host target as well as perhaps RNA-Seq studies. Also, it was suggested that to determine host interaction, the group could perform experiments in which the MOI is varied.
- This group needs to be thinking of what needs to happen for implementation of preclinical trials for lead compounds and do that. There have been no trials for CHIKV thus far, so (b) (3) (A), (b) suggested that it might be good to develop a consortia with other leaders to coordinate such trials. She suggested that (b) (3) (A), (b) (6) at Emory might be a good resource for helping to define what the first clinical trial would look like. Project 2 leaders need to start attending and being aware of FDA advisory meetings for CHIKV, even if the topic is vaccine-related, because they can shed light on what needs to be defined as far as epi endpoints: acute disease and proof of mechanism with patient population, viremia, acute clinical arthralgia severity post 3 months (clinical

including stiffness scales and pain scales). For all of this to occur, they will need to have a well thought-out clinical trial that includes consideration of drug-drug interactions. Prioritize experiments that are in line with these future goals with the lead compound.

Project 3 – Flaviviruses

Strengths: The addition of Pei-Yong Shi with his structural focus and industry experience has greatly benefitted this project. Several participants noted that the structural analysis was beautiful and was well done. (b) (3) (A), (b) (6) also brings much to this project and has provided a good approach with mouse models.

Weaknesses: Identification and development of a pan-dengue compound would be useful, but members realize that it is very difficult, due to differences in serotype, structure and mutations that increase resistance. Previously, there has been a push for project leaders to explore commercial partners within these projects, however, for this project, several larger and mid sized pharmas are independently working on Dengue antivirals because there is market for such agents. This results in there being less potential for an industry collaboration for the disease.

Future directions: One member asked if dengue is serotyped when diagnosed. It was suggested that if serotyping is done routinely, this might be a viable approach to develop therapeutics that are serotype specific even if they do not show pan-dengue efficacy.

Project 4 – Influenza

Strengths: Given the challenges that this project has faced during the first 5-year grant period, Kara Carter commented that it had made substantial progress in the past year. The HTS Core, led by Bob Bostwick has done a fantastic job and the quality of screens is very high.

Weaknesses: Even with excellent screens, it is the quality of the compounds emerging from screening that determines progress. Libraries used were limited with few hits to pursue even with a number of approaches being used. Also, IP space wasn't discussed in the presentation.

Future directions:

- Since it is still relatively early days for this project, there was a question as to how the priority to IVB was currently being considered.
- The VX787 compound (used as a positive control in studies) should be looked into further. If it has a patent, the patent information could be very informative.
- *In vivo* models for this project need to be considered. SAC suggested ferret or cotton rat models, not just the mouse models that are being used or planned.
- Because of the focus of hitting RdRp, it would make sense to perform thermal shift assays right away in order to identify hits then utilize the cap-snatch inhibitor.
- One member commented that they would like to see more studies with the EIDD 2838 compound as well as potential combination studies with what is already "out there."

Core A – Administrative
Strengths: The benefit of frequent teleconferences, team meetings and interactions among all projects and cores is clear, and has resulted in good progress for all projects.

Weaknesses: Since this was the first annual meeting under this award, new SAC members noted that clearer direction to the members prior to the meeting would be helpful in future.

Future recommendations:

- The SAC said it would have been helpful to have slide decks provided ahead of time for note taking. For the next meeting, redacted versions of the slides with the final presentations will be requested.
- Although scheduling of the meeting earlier in the year may have benefited the SAC, it was concluded that the same November meeting dates for this year should be followed next year, since they do not overlap with most other major conferences.

Core B – HTS
As noted in individual project reviews, this Core is doing excellent work in providing promising compounds for all projects. (See specific comments in the project sections above.)

Core C – Med Chem
This core is also an obvious strength of the overall program and is doing very good work. (See specific comments in the project sections above.)

IDPs:

Please refer to the project descriptions for Individual Development Plans used at respective institutions with which trainees are affiliated.

C. OVERALL PRODUCTS

C.1 PUBLICATIONS

Are there publications or manuscripts accepted for publication in a journal or other publication (e.g., book, one-time publication, monograph) during the reporting period resulting directly from this award?

Yes

Publications Reported for this Reporting Period

Public Access Compliance	Citation
Complete	Broeckel RM, Haese N, Ando T, Dmitriev I, Kreklywich CN, Powers J, Denton M, Smith P, Morrison TE, Heise M, DeFilippis V, Messaoudi I, Curiel DT, Streblow DN. Vaccine-Induced Skewing of T Cell Responses Protects Against Chikungunya Virus Disease. Frontiers in immunology. 2019;10:2563. PubMed PMID: 31736977; PubMed Central PMCID: PMC6834551; DOI: 10.3389/fimmu.2019.02563.
Complete	Leist SR, Jensen KL, Baric RS, Sheahan TP. Increasing the translation of mouse models of MERS coronavirus pathogenesis through kinetic hematological analysis. PloS one. 2019;14(7):e0220126. PubMed PMID: 31339932; PubMed Central PMCID: PMC6655769; DOI: 10.1371/journal.pone.0220126.
Complete	Li G, Adam A, Luo H, Shan C, Cao Z, Fontes-Garfias CR, Sarathy VV, Teleki C, Winkelmann ER, Liang Y, Sun J, Bourne N, Barrett ADT, Shi PY, Wang T. An attenuated Zika virus NS4B protein mutant is a potent inducer of antiviral immune responses. NPJ vaccines. 2019;4:48. PubMed PMID: 31815005; PubMed Central PMCID: PMC6883050; DOI: 10.1038/s41541-019-0143-3.
Complete	Zhang X, Xie X, Zou J, Xia H, Shan C, Chen X, Shi PY. Genetic and biochemical characterizations of Zika virus NS2A protein. Emerging microbes & infections. 2019;8(1):585-602. PubMed PMID: 30958095; PubMed Central PMCID: PMC6455252; DOI: 10.1080/22221751.2019.1598291.
Complete	Amaral JK, Taylor PC, Teixeira MM, Morrison TET, Schoen RT. The Clinical Features, Pathogenesis and Methotrexate Therapy of Chronic Chikungunya Arthritis. Viruses. 2019 March 22;11(3). PubMed PMID: 30909365; PubMed Central PMCID: PMC6466451; DOI: 10.3390/v11030289.
Complete	Broeckel R, Sarkar S, May NA, Totonchy J, Kreklywich CN, Smith P, Graves L, DeFilippis VR, Heise MT, Morrison TE, Moorman N, Streblow DN. Src Family Kinase Inhibitors Block Translation of Alphavirus Subgenomic mRNAs. Antimicrobial agents and chemotherapy. 2019 April;63(4). PubMed PMID: 30917980; PubMed Central PMCID: PMC6496153; DOI: 10.1128/AAC.02325-18.
Complete	Brown AJ, Won JJ, Graham RL, Dinnon KH 3rd, Sims AC, Feng JY, Cihlar T, Denison MR, Baric RS, Sheahan TP. Broad spectrum antiviral remdesivir inhibits human endemic and zoonotic deltacoronaviruses with a highly divergent RNA dependent RNA polymerase. Antiviral research. 2019 September;169:104541. PubMed PMID: 31233808; PubMed Central PMCID: PMC6699884; DOI: 10.1016/j.antiviral.2019.104541.
Complete	Lim XN, Shan C, Marzinek JK, Dong H, Ng TS, Ooi JSG, Fibriansah G, Wang J, Verma CS, Bond PJ, Shi PY, Lok SM. Molecular basis of dengue virus serotype 2 morphological switch from 29°C to 37°C. PLoS pathogens. 2019 September;15(9):e1007996. PubMed PMID: 31536610; PubMed Central PMCID: PMC6752767; DOI: 10.1371/journal.ppat.1007996.
Complete	Zhang X, Xie X, Xia H, Zou J, Huang L, Popov VL, Chen X, Shi PY. Zika Virus NS2A-Mediated Virion Assembly. mBio. 2019 October 29;10(5). PubMed PMID: 31662457; PubMed Central PMCID: PMC6819661; DOI: 10.1128/mBio.02375-19.
Complete	Agostini ML, Pruijssers AJ, Chappell JD, Gribble J, Lu X, Andres EL, Bluemling GR, Lockwood MA, Sheahan TP, Sims AC, Natchus MG, Saindane M, Kolykhalov AA, Painter GR, Baric RS, Denison MR. Small-Molecule Antiviral β-d-N ⁴-Hydroxycytidine Inhibits a Proofreading-Intact Coronavirus with a High Genetic Barrier to Resistance. Journal of virology. 2019 December 15;93(24). PubMed PMID: 31578288; PubMed Central PMCID: PMC6880162; DOI: 10.1128/JVI.01348-19.

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Nothing to report

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Have inventions, patent applications and/or licenses resulted from the award during the reporting period? Yes

If yes, has this information been previously provided to the PHS or to the official responsible for patent matters at the grantee organization? Yes

C.5 OTHER PRODUCTS AND RESOURCE SHARING

Nothing to report

D. OVERALL PARTICIPANTS

D.1 WHAT INDIVIDUALS HAVE WORKED ON THE PROJECT?

Commons ID	S/K	Name	Degree(s)	Role	Cal	Aca	Sum	Foreign Org	Component(s)	Country	SS
(b) (6)	Y	WHITLEY, RICHARD J.	AB,MD	PD/PI	(b) (6)	0.0	0.0		Admin Core-6072 (Administrative Core)		NA
	N	(b) (3) (A), (b) (6)		Technician		0.0	0.0		Project-6309 (Project 3.3: Develop Antiv...virus Therapy)		NA
	N		BS	Technician		0.0	0.0		Core-6075 (Core C.2 Medicinal Chemist...t Core - EIDD)		NA
	N			Technician		0.0	0.0		Project-6307 (Project 2.3: Novel Therape...ng Alphavirus)		NA
	N			Co-Investigator		0.0	0.0		Project-6079 (Project 4.1: Identify New ...a Replication)		NA
	N		PhD	Staff scientist (Doctoral level)		0.0	0.0		Project-6078 (Project 3.1: Develop Antiv...virus Therapy)		NA
	N			Technician		0.0	0.0		Project-6309 (Project 3.3: Develop Antiv...virus Therapy)		NA
	N			Technician		0.0	0.0		Project-6307 (Project 2.3: Novel Therape...ng Alphavirus)		NA
	N			Technician		0.0	0.0		Project-6309 (Project 3.3: Develop Antiv...virus Therapy)		NA
	N			Technician		0.0	0.0		Project-6078 (Project 3.1: Develop Antiv...virus Therapy)		NA
	N		PhD	Staff scientist (Doctoral level)		0.0	0.0		Project-6078 (Project 3.1: Develop Antiv...virus		NA

									Therapy)		
(b) (6)	Y	(b) (3) (A), (b) (6)	PhD	Co-Investigator	(b) (6)	0.0	0.0		Project-6301 (Project 1.2: The Developme…s Replication)		NA
	N		PhD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position		0.0	0.0		Project-6077 (Project 2.1: Novel Therape…ng Alphavirus)		NA
	N		PHD,BS, MS	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position		0.0	0.0		Project-6077 (Project 2.1: Novel Therape…ng Alphavirus)		NA
	Y		PhD	Co-Investigator		0.0	0.0		Core-6074 (Core C.1 Medicinal Chemist…ent Core - SR)		NA
	Y		MD, PhD	Co-Investigator		0.0	0.0		Project-6076 (Project 1.1: The Developme…s Replication)		NA
	Y		PHD,MS	Co-Investigator		0.0	0.0		Project-6077 (Project 2.1: Novel Therape…ng Alphavirus)		NA
	N		BS	Graduate Student (research assistant)		0.0	0.0		Project-6303 (Project 2.2: Novel Therape…ng Alphavirus)		NA
	N		PhD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position		0.0	0.0		Project-6310 (Project 4.2: Identify New …a Replication)		NA
	Y		PhD	Co-Investigator		0.0	0.0		Core-6075 (Core C.2 Medicinal Chemist…t Core - EIDD)		NA
	N		PHD,BA	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position		0.0	0.0		Project-6077 (Project 2.1: Novel Therape…ng Alphavirus)		NA
	Y		BA,PHD	Co-Investigator		0.0	0.0		Project-6311 (Project 3.2: Develop Antiv…virus		NA

									Therapy)		
(b) (6)	N	(b) (3) (A), (b) (6)	PhD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position	(b) (6)	0.0	0.0		Project-6078 (Project 3.1: Develop Antiv…virus Therapy)		NA
	N		PHD,BS	Faculty		0.0	0.0		Project-6311 (Project 3.2: Develop Antiv…virus Therapy)		NA
	Y		PhD	Co-Investigator		0.0	0.0		Core-6074 (Core C.1 Medicinal Chemist…ent Core - SR)		NA
	N			Postdoctoral Scholar, Fellow, or Other Postdoctoral Position		0.0	0.0		Project-6309 (Project 3.3: Develop Antiv…virus Therapy)		NA
	Y		MS,PHD	Co-Investigator		0.0	0.0		Project-6076 (Project 1.1: The Developme…s Replication)		NA
	Y		BS,PHD	Co-Investigator		0.0	0.0		Project-6301 (Project 1.2: The Developme…s Replication)		NA
	N		PhD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position		0.0	0.0		Project-6303 (Project 2.2: Novel Therape…ng Alphavirus)		NA
	Y		PHD,BS	Co-Investigator		0.0	0.0		Project-6301 (Project 1.2: The Developme…s Replication)		NA
	N		PhD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position		0.0	0.0		Project-6078 (Project 3.1: Develop Antiv…virus Therapy)		NA
	N		PhD	Assistant Staff Scientist		0.0	0.0		Project-6077 (Project 2.1: Novel Therape…ng Alphavirus)		NA
	N		PhD	Chemist		0.0	0.0		Core-6074 (Core C.1 Medicinal Chemist…ent		

		(b) (3) (A), (b) (6)			(b) (6)				Core - SR)		
	N			Adj. Research Associate Professor		0.0	0.0		Project-6077 (Project 2.1: Novel Therape…ng Alphavirus)		NA
	N			Laboratory Assistant		0.0	0.0		Project-6309 (Project 3.3: Develop Antiv…virus Therapy)		NA
	N		PhD	Senior Research Scientist		0.0	0.0		Core-6074 (Core C.1 Medicinal Chemist…ent Core - SR)		NA
	Y			Program Manager		0.0	0.0		Admin Core-6072 (Administrative Core)		NA
	N		MS	Chemist		0.0	0.0		Core-6074 (Core C.1 Medicinal Chemist…ent Core - SR)		NA
	N		BS	Research Specialist		0.0	0.0		Project-6301 (Project 1.2: The Developme…s Replication)		NA
	N			Supervisor, Compound Management		0.0	0.0		Core-6073 (Assay Core)		NA
	N		PhD	Researcher		0.0	0.0		Core-6075 (Core C.2 Medicinal Chemist…t Core - EIDD)		NA
	N			Program Coordinator		0.0	0.0		Admin Core-6072 (Administrative Core)		NA
	N		PhD	Chemist		0.0	0.0		Core-6074 (Core C.1 Medicinal Chemist…ent Core - SR)		NA
	N			Senior Research Assistant		0.0	0.0		Project-6077 (Project 2.1: Novel Therape…ng Alphavirus)		NA
	N			Research Technician		0.0	0.0		Project-6079 (Project 4.1: Identify New …a Replication)		NA

	N	(b) (3) (A), (b) (6)	MS	Project Manager	(b) (6)	0.0	0.0		Core-6074 (Core C.1 Medicinal Chemist...ent Core - SR)		NA
	Y			Assistant Director		0.0	0.0		Admin Core-6072 (Administrative Core)		NA
	N		PhD	Chemist		0.0	0.0		Core-6074 (Core C.1 Medicinal Chemist...ent Core - SR)		NA
	N			Research Assistant 2		0.0	0.0		Project-6311 (Project 3.2: Develop Antiv...virus Therapy)		NA
	N			Chemist		0.0	0.0		Core-6074 (Core C.1 Medicinal Chemist...ent Core - SR)		NA
	N			Senior Research Specialist		0.0	0.0		Project-6076 (Project 1.1: The Developme...s Replication)		NA
	N			Laboratory Supervisor		0.0	0.0		Project-6079 (Project 4.1: Identify New ...a Replication)		NA
	N			Senior Research Assistant		0.0	0.0				NA
	N			Senior Research Specialist		0.0	0.0		Project-6076 (Project 1.1: The Developme...s Replication)		NA
	N		BS	Research Specialist		0.0	0.0		Project-6301 (Project 1.2: The Developme...s Replication)		NA
	N			Data Analyst 2		0.0	0.0		Core-6073 (Assay Core)		NA
	N		MS	Biologist		0.0	0.0		Project-6310 (Project 4.2: Identify New ...a Replication)		NA
	N			Senior Research Assistant		0.0	0.0		Project-6077 (Project 2.1: Novel Therape...ng		NA

									Alphavirus)		
(b) (6)	N	(b) (3) (A), (b) (6)	PhD	Senior Research Scientist	(b) (6)	0.0	0.0		Core-6074 (Core C.1 Medicinal Chemist…ent Core - SR)		NA
	N		PhD	Research Chemist		0.0	0.0		Core-6074 (Core C.1 Medicinal Chemist…ent Core - SR)		NA
	N			Scientist 3		0.0	0.0		Core-6073 (Assay Core)		NA
	N			Research Associate		0.0	0.0		Project-6311 (Project 3.2: Develop Antiv…virus Therapy)		NA
	N			Senior Research Assistant		0.0	0.0		Project-6077 (Project 2.1: Novel Therape…ng Alphavirus)		NA
	N		PhD	Research Scientist		0.0	0.0		Project-6301 (Project 1.2: The Developme…s Replication)		NA
	N		MS	Chemist		0.0	0.0		Core-6074 (Core C.1 Medicinal Chemist…ent Core - SR)		NA
	N			Scientist 3		0.0	0.0		Core-6073 (Assay Core)		NA
	N			Chemist		0.0	0.0		Core-6074 (Core C.1 Medicinal Chemist…ent Core - SR)		NA
	N		PhD	Chemist		0.0	0.0		Core-6074 (Core C.1 Medicinal Chemist…ent Core - SR)		NA
	N		PhD	Researcher		0.0	0.0		Core-6075 (Core C.2 Medicinal Chemist…t Core - EIDD)		NA
	Y		PhD	Site Principal Investigator		0.0	0.0		Project-6310 (Project 4.2: Identify New …a Replication)		NA
	Y	Denison,	MD	Project Lead		0.0	0.0		Project-6076		NA

(b) (6)		Mark							(Project 1.1: The Developme…s Replication)		
	N	(b) (3) (A), (b) (6)	PhD	Researcher	(b) (6)	0.0	0.0		Core-6075 (Core C.2 Medicinal Chemist…t Core - EIDD)		NA
	Y		PHD	Site Principal Investigator		0.0	0.0		Core-6075 (Core C.2 Medicinal Chemist…t Core - EIDD)		NA
	Y		PhD	Co-Site Principal Investigator		0.0	0.0		Project-6303 (Project 2.2: Novel Therape…ng Alphavirus)		NA
	N			Instructor		0.0	0.0		Project-6309 (Project 3.3: Develop Antiv…virus Therapy)		NA
	Y	Bostwick, Robert		Site Principal Investigator		0.0	0.0		Core-6073 (Assay Core)		NA
	Y	(b) (3) (A), (b) (6)	BS,BS,MS	Associate Director, HTS Center		0.0	0.0		Core-6073 (Assay Core)		NA
	Y		MD, PhD	Site Principal Investigator		0.0	0.0		Project-6309 (Project 3.3: Develop Antiv…virus Therapy)		NA
	N		PhD	Researcher		0.0	0.0		Core-6075 (Core C.2 Medicinal Chemist…t Core - EIDD)		NA
	N		PhD	Researcher		0.0	0.0		Core-6075 (Core C.2 Medicinal Chemist…t Core - EIDD)		NA
	Y			Site Principal Investigator		0.0	0.0		Project-6311 (Project 3.2: Develop Antiv…virus Therapy)		NA
	Y	Pathak, Ashish K	PhD	Project Leader		0.0	0.0		Core-6074 (Core C.1 Medicinal Chemist…ent Core - SR)		NA
	Y	SHI, PEI-YONG	PHD	Site Project Leader		0.0	0.0		Project-6078 (Project 3.1: Develop Antiv…virus		NA

									Therapy)		
(b) (6)	Y	(b) (3) (A), (b) (6)	BS,PHD	Investigator	(b) (6)	0.0	0.0		Project-6301 (Project 1.2: The Developme...s Replication)		NA
	Y		PhD	Site Principal Investigator		0.0	0.0		Project-6301 (Project 1.2: The Developme...s Replication)		NA
	Y	Whitley, Richard		Site Principal Investigator		0.0	0.0		Project-6079 (Project 4.1: Identify New ...a Replication)		NA
	N	(b) (3) (A), (b) (6)	PhD	Research Associate		0.0	0.0		Project-6303 (Project 2.2: Novel Therape...ng Alphavirus)		NA
	Y			Research Associate		0.0	0.0		Project-6301 (Project 1.2: The Developme...s Replication)		NA
	N		PhD	Scientist		0.0	0.0		Core-6074 (Core C.1 Medicinal Chemist...ent Core - SR)		NA
	N		PhD	Chemist		0.0	0.0		Core-6074 (Core C.1 Medicinal Chemist...ent Core - SR)		NA
	Y	STREBLOW, DANIEL N	PHD,BS	Site Project Leader		0.0	0.0		Project-6077 (Project 2.1: Novel Therape...ng Alphavirus)		NA
	Y	(b) (3) (A), (b) (6)	BA,BA,MA ,PHD	Site Principal Investigator		0.0	0.0		Project-6307 (Project 2.3: Novel Therape...ng Alphavirus)		NA

Glossary of acronyms:
S/K - Senior/Key
DOB - Date of Birth
Cal - Person Months (Calendar)
Aca - Person Months (Academic)
Sum - Person Months (Summer)
Foreign Org - Foreign Organization Affiliation
SS - Supplement Support
RE - Reentry Supplement
DI - Diversity Supplement
OT - Other
NA - Not Applicable

D.2 PERSONNEL UPDATES

D.2.a Level of Effort

Will there be, in the next budget period, either (1) a reduction of 25% or more in the level of effort from what was approved by the agency

for the PD/PI(s) or other senior/key personnel designated in the Notice of Award, or (2) a reduction in the level of effort below the minimum amount of effort required by the Notice of Award?

Yes

The level of effort for (b) (3) (A), (b) (6) from project 4 has been decreased from 20% to 5% in grant year 2. This reduction was made due to reformatting the size of the animal studies to be completed in grant year 2 and the 16.7% reduction to the proposed budget for this project. The Project 4 team has also decided to remove (b) (3) (A), from the grant year 2 key personnel. The team will be utilizing (b) (b) (3) (A), in vivo influenza models on an as needed basis, and he will not contribute a consistent percent effort to the grant throughout the year. (b) (3) (A), has provided the team a cost per animal study going forward, which will be used for budgeting purposes.

D.2.b New Senior/Key Personnel

Are there, or will there be, new senior/key personnel?

Yes

File uploaded: (b) (3) (A), _Biosketch_v20191218.pdf

D.2.c Changes in Other Support

Has there been a change in the active other support of senior/key personnel since the last reporting period?

Yes

File uploaded: AD3C All Combined OS changes Y1.pdf

D.2.d New Other Significant Contributors

Are there, or will there be, new other significant contributors?

No

D.2.e Multi-PI (MPI) Leadership Plan

Will there be a change in the MPI Leadership Plan for the next budget period?

NA

OMB No. 0925-0001 and 0925-0002 (Rev. 09/17 Approved Through 03/31/2020)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. DO NOT EXCEED FIVE PAGES.

NAME: (b) (3) (A), (b) (6)

eRA COMMONS USER NAME (credential, e.g., agency login): (b) (6)

POSITION TITLE: (b) (3) (A), (b) (6) Antiviral Drug Discovery and Development Center

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE (if applicable)	END DATE MM/YYYY	FIELD OF STUDY
(b) (3) (A), (b) (6)	BS PHD	(b) (3) (A), (b) (6)	Chemistry Pathology

A. Personal Statement

I am excited to join the team of the CETR U19 award Alabama Drug, Development, and Discovery Coalition (AD3C). My interest and skills closely align with management of multiple projects and facilitation of the success of essential collaborations. It is exciting to support new ideas and projects and to know this position ensures the continued success of these ventures. I get great satisfaction and am adept at working in a team environment toward a common goal. Over the past few years I have honed my skills towards project management through the (b) (3) (A), (b) (6). In addition, in my previous postdoctoral fellowship, I've gained experience overseeing multiple projects including the management of 1 high school student, 4 undergraduates, 2 medical students, 3 residents, and 2 fellows. I performed these tasks while also successfully working on my personal and collaborative projects. For each of these projects, I have had to evaluate the timeline and the breadth of the endeavor to then decide the goals for that project. I experience in designing and performing experiments for publication and presentation. In addition to analyzing and interpreting all data collected for each of these projects with trainees, I have also built programs and led workshops for junior researchers on topics such as preparation of an NIH biosketch, personal statement, and mental health and wellness. As a member of the administrative core of AD3C, I am committed to being reliable and facilitating progress and communication among all groups of the consortium.

B. Positions and Honors

(b) (3) (A), (b) (6)

Other Experience and Professional Memberships

(b) (3) (A), (b) (6)

Honors

(b) (3) (A), (b) (6)

C. Contribution to Science

(b) (3) (A), (b) (6)


(b) (3) (A), (b) (6)

OTHER SUPPORT

(b) (3) (A), (b) (6)

ACTIVE

(b) (3) (A), (b) (6), (b) (4)

OTHER SUPPORT

DENISON, MARK R.

ACTIVE

5R01AI108197-07 Denison/Baric 08/01/2013-02/28/2023 (b) (6) calendar months
NIAID/NIH $532,971
Determinants of Coronavirus Fidelity in Replication and Pathogenesis
Experiments in this aim will test the hypothesis nsp1 functions in maintaining high replication fidelity and viral RNA synthesis are coupled and that targeted engineered mutations across nsp14 alter: a) RNA fidelity outcomes; b) sensitivity nucleoside mutagens, terminators and polymerase inhibitors; c) the kinetics and magnitude of positive, negative, genomic and subgenomic RNA synthesis; and d) RNA recombination frequencies.

5K12HD087023-06 Webber 12/01/2015-11/30/2020 (b) (6) calendar months
NIAID/NIH $320,112
Pathogenesis, Targeted Therapeutics, and New Vaccines for Childhood Disease
The goal of this project is to establish at Vanderbilt School of Medicine a mentored training program for young pediatricians possessing both the aptitude and passion to become a new generation of basic and translational physician scientists. Dr. Denison serves as a training director for the program.

VUMC63419 (1R01AI132178-02) Baric 08/09/2017-07/31/2022 (b) (6) calendar months
UNC/NIAID/NIH $200,000
Broad-spectrum antiviral GS-5734 to treat MERS-CoV and related emerging CoV
The goal of this project is to define the efficacy, pharmacokinetics, mechanism, resistance and genetics of susceptibility of known and potentially emerging coronaviruses to the nucleoside prodrug GS-5734 in vitro and in vivo. The VUMC team will focus on studies to define the mechanism of GS-5734 in vitro against SARS-CoV and MERS-Cov.

5T32AI095202-10 Denison 08/18/2016-07/31/2021 (b) (6) calendar months
NIAID/NIH $284,247
Childhood Infections Research Program (CHIRP)
The training grant is designed to train and facilitate the careers of investigators studying infectious diseases relevant to children. The outcomes will be both MD and PhD researchers capable of making a long-term impact on the diagnosis, prevention, and treatment of infections in children. The public health relevance is high for the direct impact on the health of children.

1U19AI142759-01 Whitley 03/07/2019-02/29/2024 (b) (6) calendar months
NIAID/NIH $283,186
Antiviral Drug Discovery and Development Center
Zoonotic coronaviruses (CoVs) such as SARS-CoV and MERS-CoV are pandemic threats, with MERS-CoV continuing to cause new zoonotic and human transmission and illness with ~35% mortality. The overall goal of our program is to develop CoV antiviral strategies that broadly inhibit known and future potential pandemic zoonotic CoVs, prevent emergence of resistance, and extend the therapeutic window by targeting host immunopathologic responses.

VUMC76604 (75N91019D00024) Chappell 09/01/2019-08/30/2020 (b) (6) calendar months
NIH-VRC (b) (6), (b) (4) (17X198) $166,330
Development of Active and Passive Vaccination Strategies for Middle East Respiratory Syndrome Based on Spike Protein
The goal of this project is to advance MERS-CoV countermeasures development by informing the rational design of efficacious 1) active vaccines based on native S trimers and 2) passive vaccines comprised of mAb combinations that target multiple S neutralizing epitopes, thereby instrumentally contributing to a preclinical scientific package qualifying candidate MERS-CoV vaccines for human testing.

OTHER SUPPORT - OREGON HEALTH & SCIENCE UNIVERSITY

STREBLOW, DN

ACTIVE

5 R01 AI116633-03 (Streblow) 3/01/2016 – 2/28/2021 (b) (6) calendar
NIH/NIAID $2,187,500
Characterizing the Role of CMV Latency in Solid Organ Transplant Rejection
The main goal of this project is to determine the mechanisms of cytomegalovirus-mediated solid organ transplant rejection.

(THIS AWARD)
1 U19 AI142759-01 (Whitley) 3/1/2019 – 2/29/2024 (b) (6) calendar
NIH/NIAID $2,567,911 – Project 2 only
Antiviral Drug Discovery and Development Center: Project 2: Novel Therapeutics for Emerging Alphavirus
The purpose of the center is to discover potential new drugs which could be used to treat infections such as West Nile virus and influenza that routinely infect US citizens, and for which we have limited or no treatments. We will also strive to develop therapies for emerging infections such as coronaviruses, dengue and chikungunya, which pose risks for traveling US citizens or could be imported into the country by others.
Role: Project Lead, Project 2

5 U19 AI128741-03 (Picker) 3/02/2017 – 2/28/2022 (b) (6) calendar
NIH/NIAID $11,807,928
Development of Immunogenicity- and Efficacy-Optimized CMV Vectors for an HIV/AIDS Vaccine: Project 4: Optimization of CMV Vector CD8+ T Cell Response Programming by Modification of CMV-Encoded Molecular Mechanisms That Interfere with Unconventional CD8+ T Cell Response Priming
In Project 4 we first seek to identify the specific motifs in the UL128 and UL130 proteins which block MHC-E-restricted CD8+ T cell priming with the goal of mutating these regions to abrogate their MHC-E-response inhibition while retaining PRC function. Since MHC-E-restricted response priming is myeloid cell-dependent, whereas MHC-II responses are disfavored by vector infection of myeloid cells, this should result in a vector that preferentially elicits MHC-E-restricted responses. Second, since HCMV lacking UL128 and UL130 fails to elicit unconventional T cell responses in RM, it is possible that HCMV encodes other genes that block unconventional CD8+ T cell responses. Therefore, utilizing a deletion strategy we will ascertain whether any non-essential HCMV genes have this activity. Finally, utilizing the data from Projects 2-4, Project 4 will construct the 2nd generation "response and safety" optimized HCMV/HIV vector for manufacture and clinical testing in Project 5. Role: Project Leader, Project 4

5 P01 AI127335-02 (Nelson/Yurochko) 8/15/2017 – 7/31/2022 (b) (6) calendar
NIH/NIAID $9,267,317
Human Cytomegalovirus Dysregulation of Host Hematopoietic Progenitor Cell Signaling Pathways to Modulate Latency, Reactivation and Hematopoiesis during Transplantation: Project 3: HCMV US28 regulation of host cell signaling in viral latency and hematopoiesis
In this project we will examine UL7 signaling in CD34+ HPCs and identify the cellular pathways necessary for viral reactivation and hematopoiesis in the context of infection. We will also determine whether these UL7 pathways cross-talk with pathways modulated by UL133/8 (Project 1), HCMV miRNAs (Project 2), US28 (Project 3) and wild-type HCMV infection (Project 5). Role: Project Leader, Project 3

1 R01 AI143660-01 (DeFilippis) 4/01/2019 – 3/31/2024 (b) (6) calendar
NIH/NIAID $3,685,293
Mechanistic Exploration of cGAS-STING-Mediated Vaccine Enhancement
The major goal of this project is to characterize the molecular, cellular, and immunological bases of adaptive immune responses augmented by the innate protein STING.
Role: Co-Investigator

(NEW)
1 R41 AI138964-01A1 (Streblow) 3/20/2019 – 2/29/2020 (b) (6) calendar
NIH/NIAID $135,676
Biologically Targeted Vaccine for Chikungunya
The major goal of this project is to develop adenoviral vaccines against chikungunya virus using novel targeting methods
Role: Subcontract PI

(b) (6), (b) (4) (Picker) 7/28/2014 – 10/31/2020 (b) (6) calendar
$28,967,114
Development of Attenuated CMV Vectors for an HIV/AIDS Vaccine
The overall goal of this project is the development of an HCMV vector-based HIV/AIDS vaccine (composed of one or more HIV insert-expressing HCMV vectors) that is optimized for safety, efficacy and manufacturability.
Role: Project Manager; Outcome 5

(NEW)
1 R01 HD096741-01A1 (Streblow/Frias) 6/01/2019 – 5/31/2024 (b) (6) calendar
NIH/NIAID $3,340,446
Mechanistic Analysis of Zika Virus Induced Placental Damage During Pregnancy
The major goal of this project is to identify the mechanisms of Zika virus induced placental damage during pregnancy.
Role: PI

Departmental Support (b) (6) calendar
Vaccine and Gene Therapy Institute

OVERLAP
No active or pending application has scientific or budgetary overlap. If a pending grant is awarded, effort will be adjusted between the OHSU support, existing grants and the new grant, following NIH guidelines.

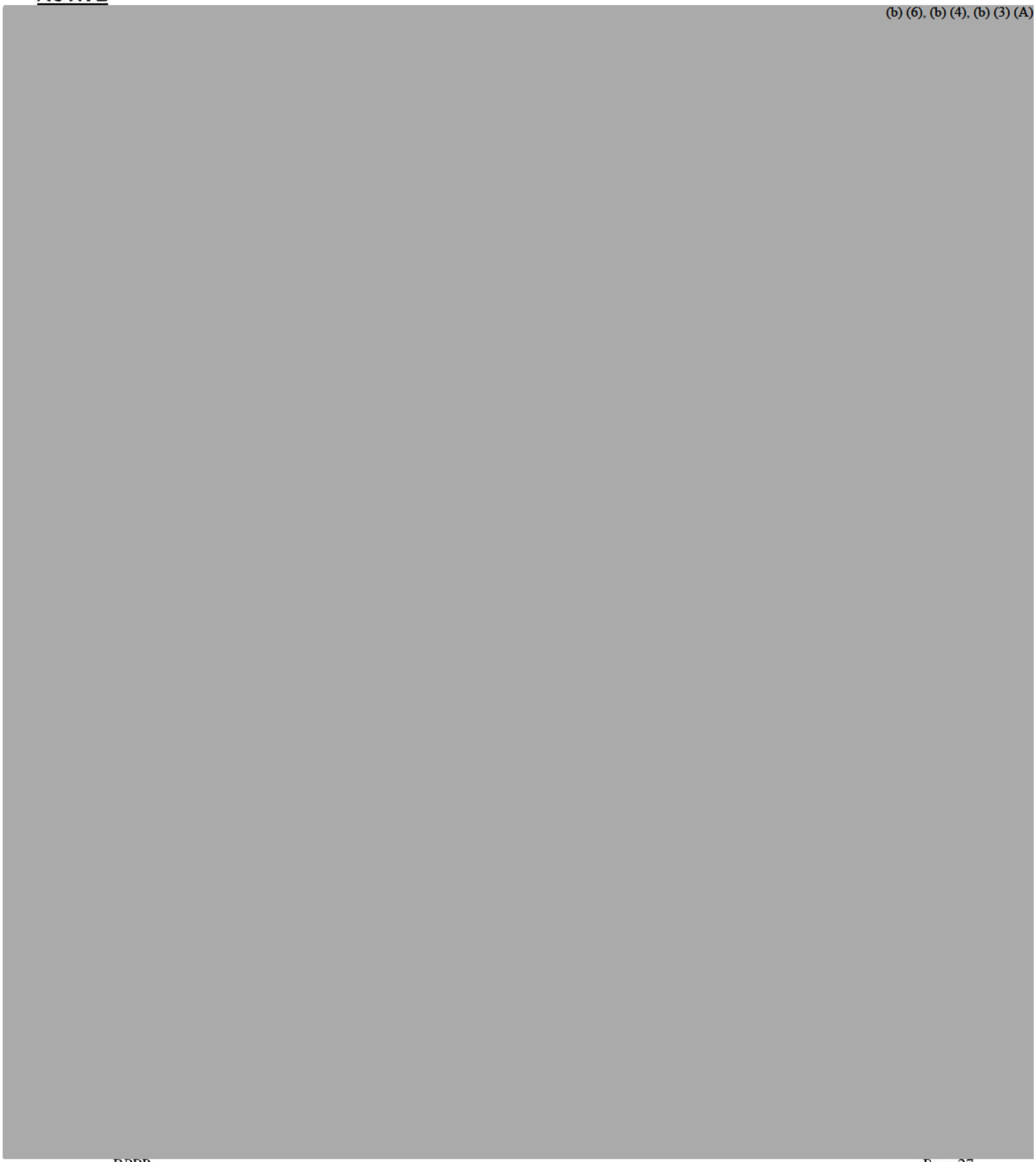
INACTIVE
5 U19 AI109680-05 (Whitley) 3/01/2014 – 2/28/2019 (b) (6) calendar
Univ. Alabama, Birmingham / NIH/NIAID $299,200
Antiviral Drug Discovery and Development Center`
Project 3B: Novel Therapeutic Strategies Targeting Re-emerging Alphaviruses
The main goal of this project is to develop novel nucleoside and nucleotide inhibitors directed against Alphaviruses including Chikungunya virus and Venezuelan Equine Encephalitis virus.
Role: Project Leader; Project 3B

OTHER SUPPORT – OREGON HEALTH & SCIENCE UNIVERSITY

(b) (6), (b) (3) (A)

ACTIVE

(b) (6), (b) (4), (b) (3) (A)




(b) (6), (b) (4), (b) (3) (A)

OTHER SUPPORT

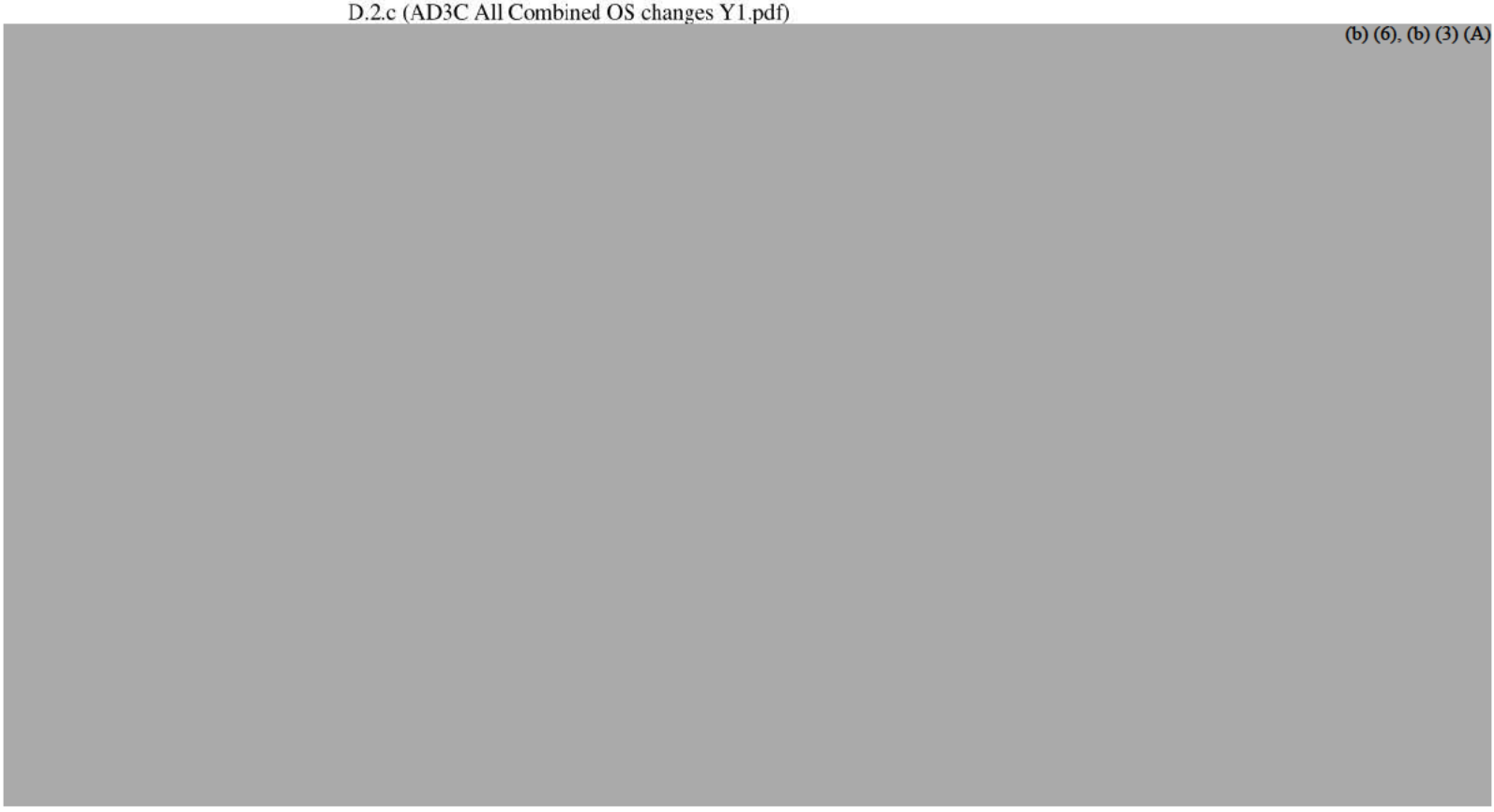
(b) (6), (b) (3) (A)

ACTIVE RESEARCH SUPPORT

(b) (6), (b) (3) (A)

D.2.c (AD3C All Combined OS changes Y1.pdf)

(b) (6), (b) (3) (A)

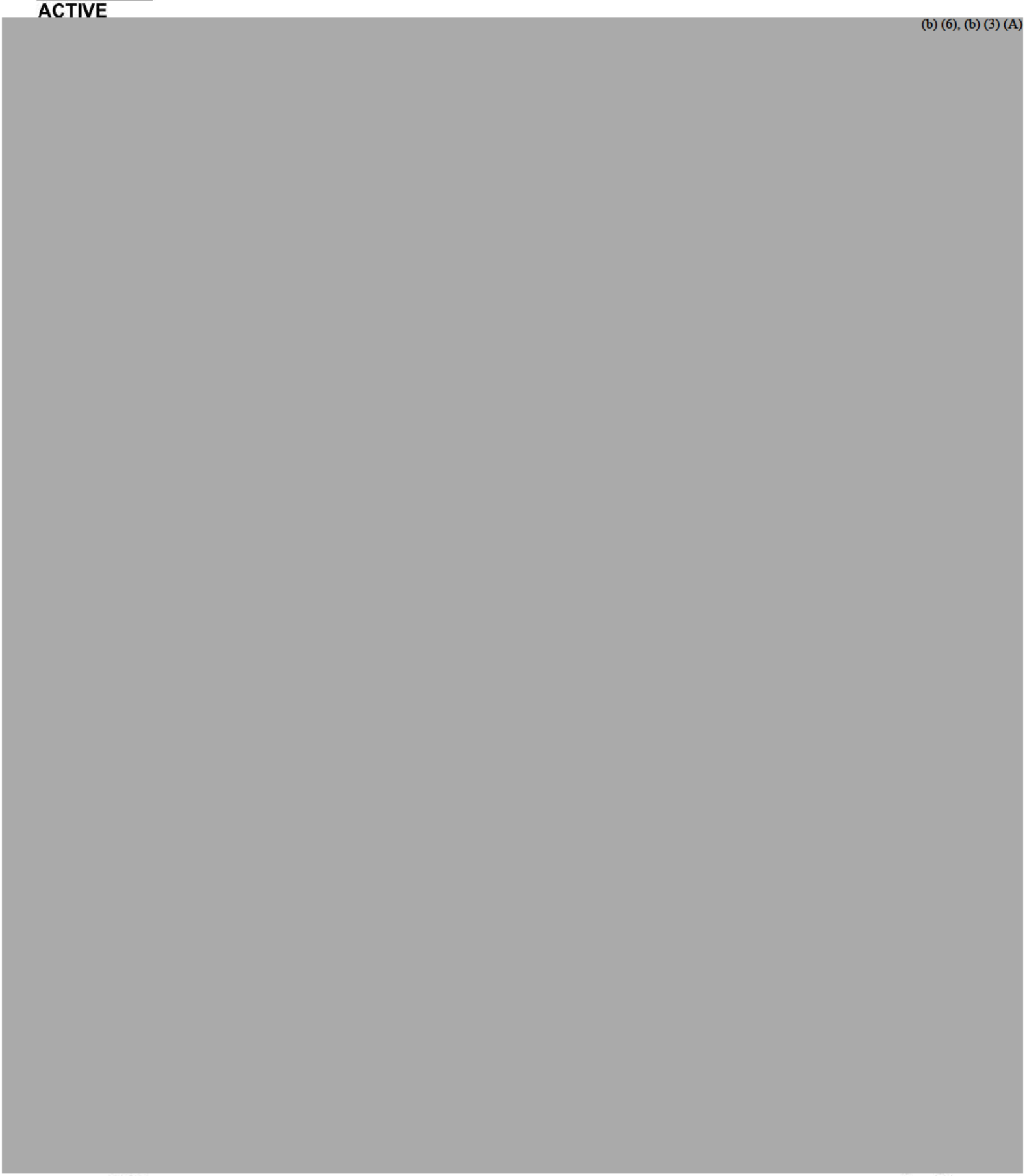


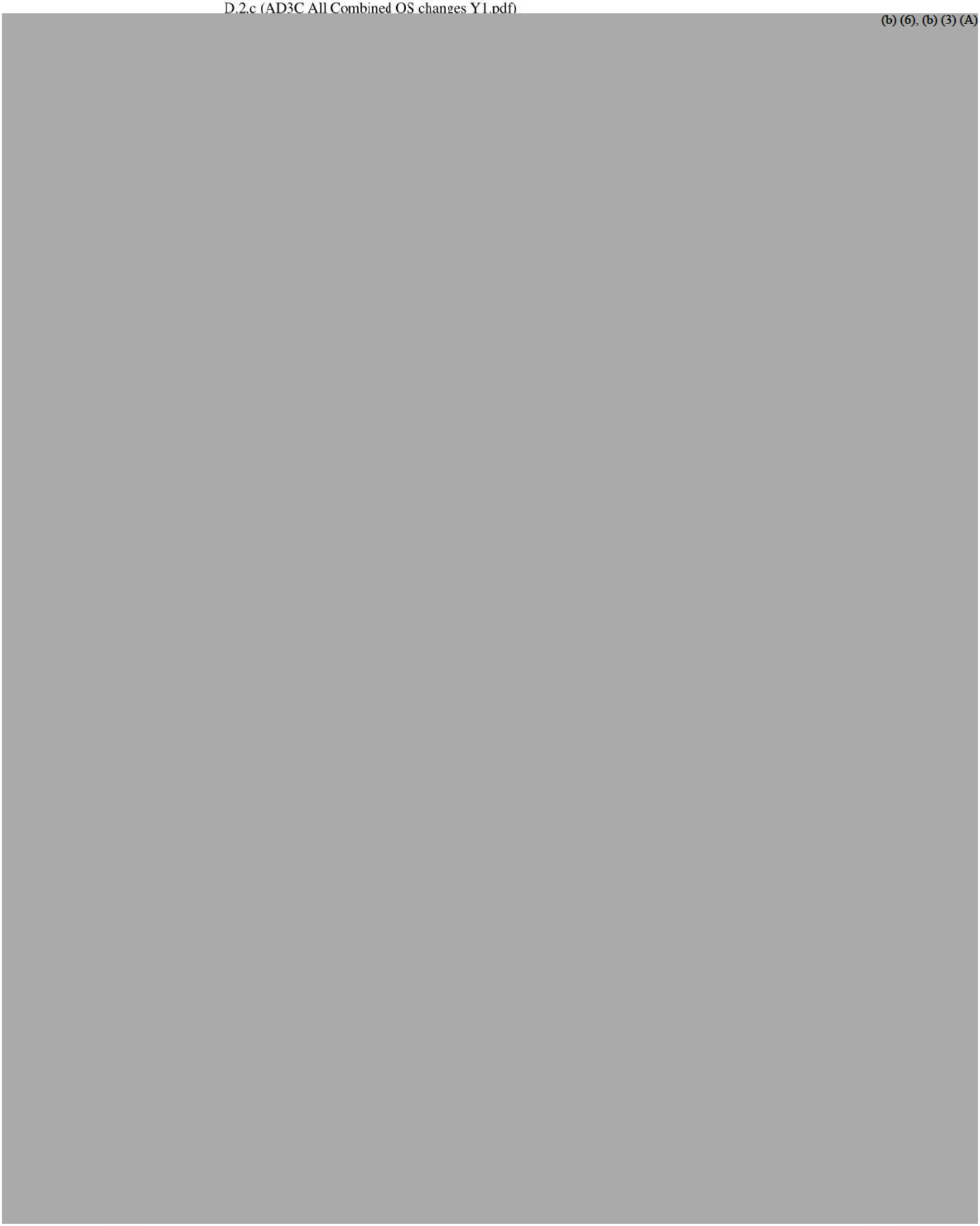
OTHER SUPPORT – OREGON HEALTH & SCIENCE UNIVERSITY

(b) (6), (b) (3) (A)

ACTIVE

(b) (6), (b) (3) (A)




(b) (6), (b) (3) (A)

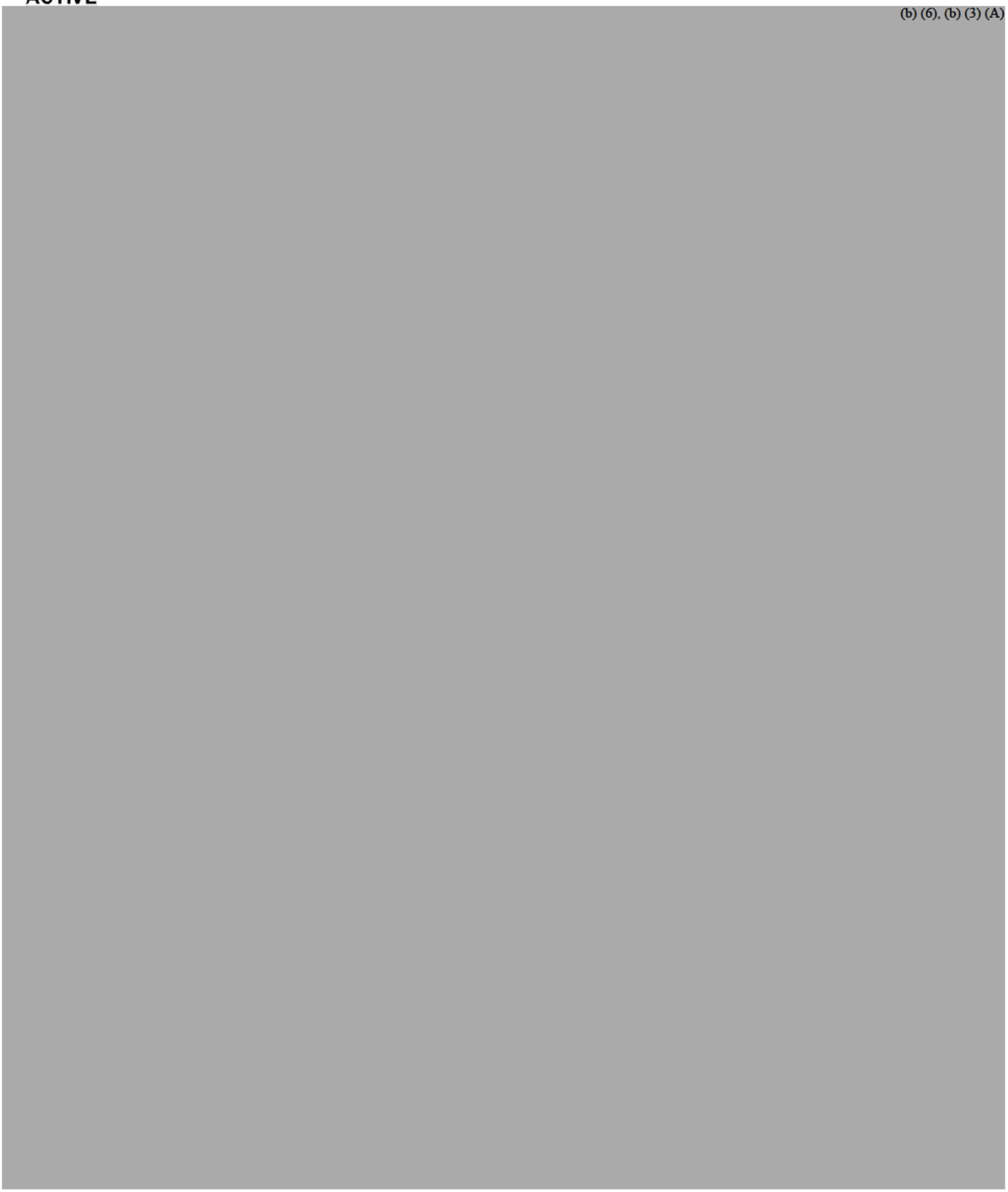

(b) (6), (b) (3) (A)

OTHER SUPPORT FOR ALL KEY PERSONNEL– OREGON HEALTH & SCIENCE UNIVERSITY

(b) (6), (b) (3) (A)

ACTIVE

(b) (6), (b) (3) (A)

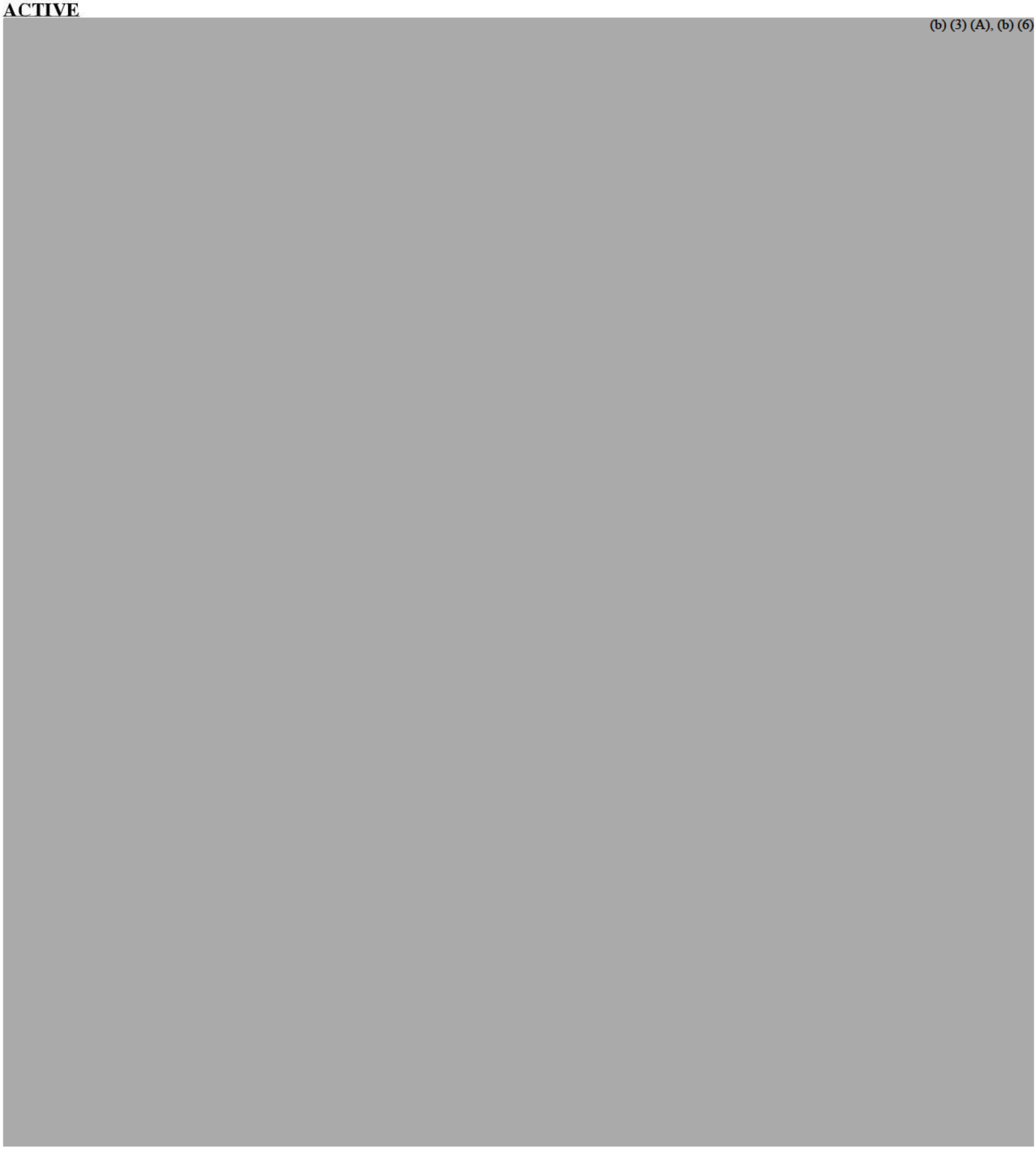


OTHER SUPPORT

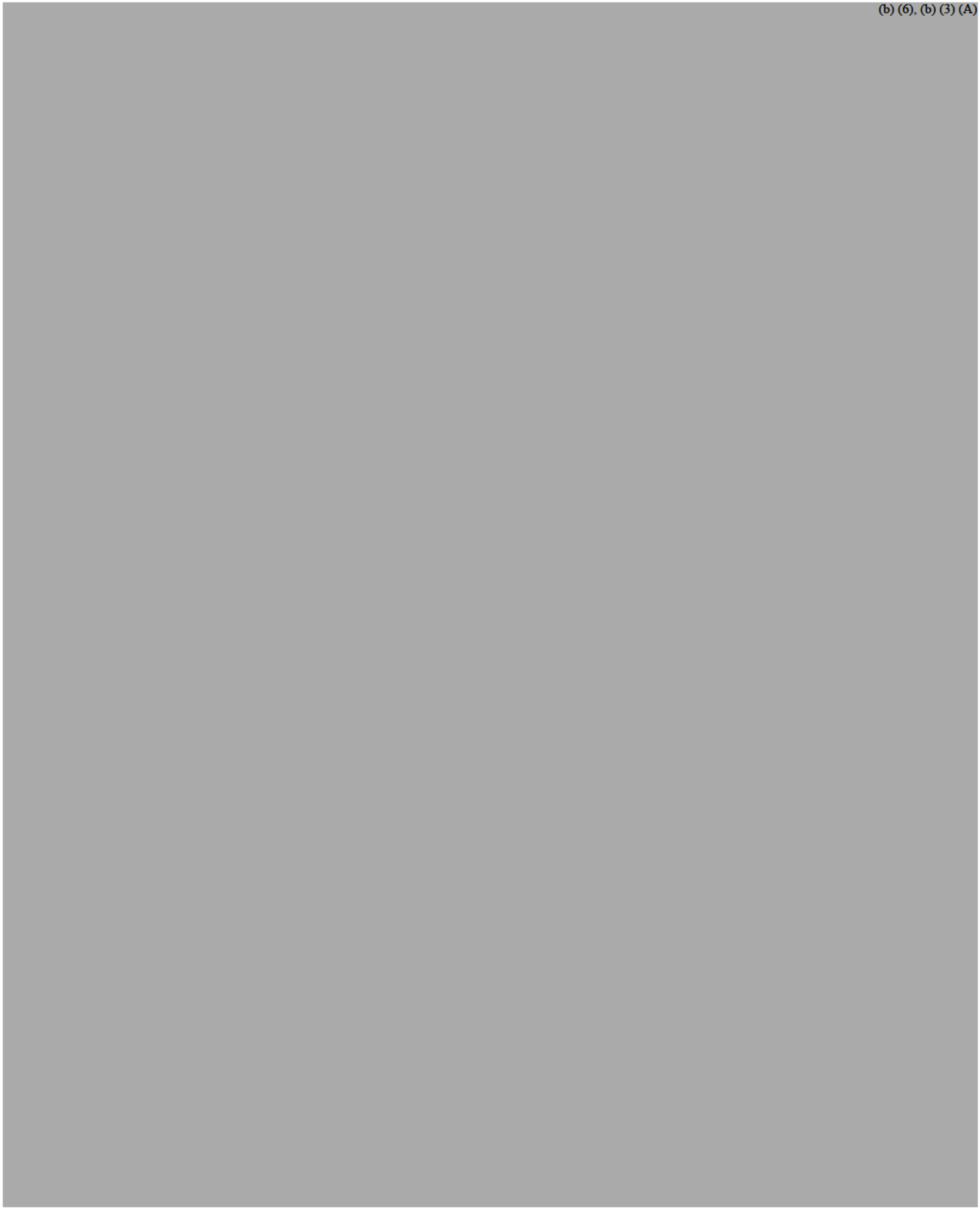
(b) (3) (A), (b) (6)

ACTIVE

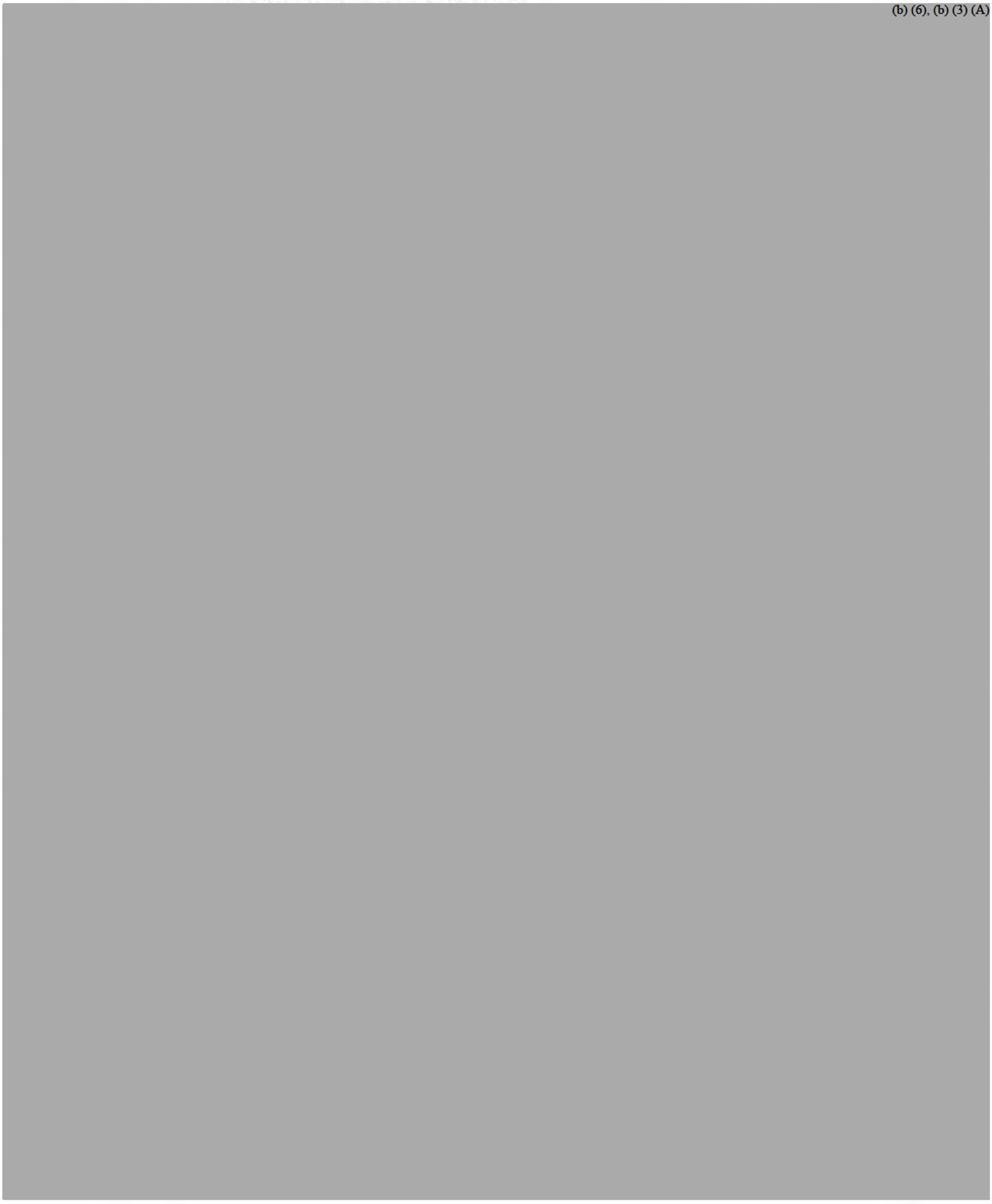
(b) (3) (A), (b) (6)



(b) (6), (b) (3) (A)

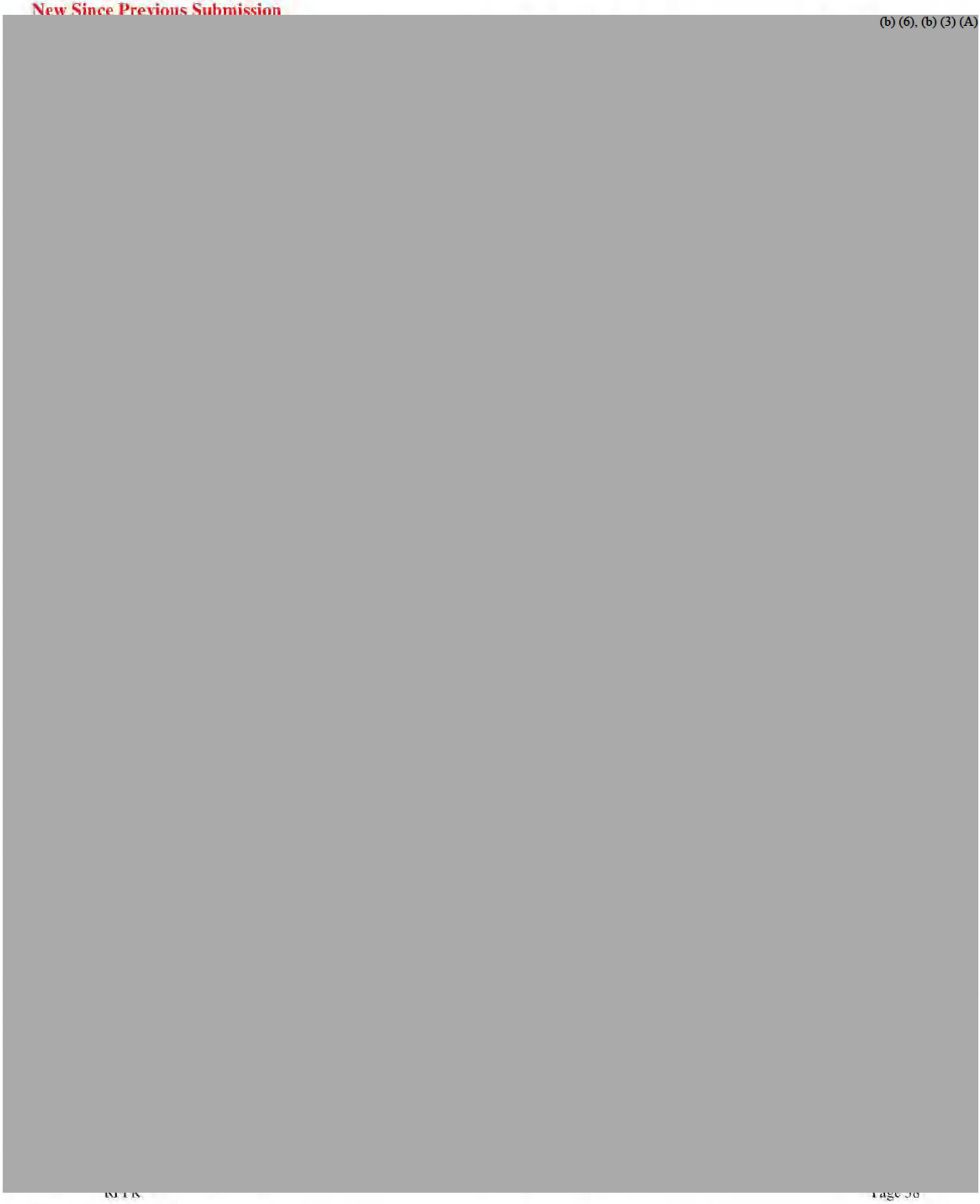


(b) (6), (b) (3) (A)



New Since Previous Submission

(b) (6), (b) (3) (A)



D.2.c (AD3C All Combined OS changes Y1.pdf)

(b) (6), (b) (3) (A)



E. OVERALL IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

NOTHING TO REPORT

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

Not Applicable

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?

NOTHING TO REPORT

F. OVERALL CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

Overall Section F.2 CETR required: Actual or anticipated challenges or delays and actions or plans to resolve them Provide a narrative that addresses the following points:

Part 1. What changes, if any, have occurred in the last year to the structure of the CETR in terms of key objectives, activities, and/or personnel?
Key objectives remain the same for all projects and cores.
Activities continue as expected.
Personnel changes have occurred as follows, but have been resolved to maintain overall structure and function of each component:
Core A: During the Y1 reporting period, the administrative core replaced (b) (6), (b) (3) (A) with (b) (6), (b) (3) (A). The change has been managed via the continuous attention to organization that the administrative core had and continues to maintain. We do not anticipate personnel changes to result in problems for Y2 and expect only continue to perform core A functions accordingly. (b) (6), (b) (3) biosketch has been added in section D.2.
Project 4: Changes to key personnel were previously approved for this project after the passing of Dr. Mark Prichard. (b) (6), (b) (3) (A) will serve as co-investigators. Also, as indicated in section D.1 (b) (6), (b) (3) (A) will be removed as a key personnel so that his service may be used on a contract basis as funds were limited. It is expected that these changes will promote the completion of project specific milestones and stay on budget.

Part 2. Discuss the Go/No-Go status of each Research Project and Core.
Core A:
The administrative core helps to prioritize focus based on input from the scientific advisory committee and facilitate connection with others in the project to give insight regarding go/no-go decisions.

Core B:
In order to identify additional chemical structures for development of new leads in projects 3 and 4, we conducted HTS of a 200K ChemDiv compound library. The compounds in this collection are structurally diverse from the compounds in the Enamine and CHemBridge libraries previously screened for these projects. The immunofluorescence assays employed as the SAR driving assays in these projects were used in HTS as opposed to a CPE assay previously used for Dengue and an enzyme linked virus reporter assay (ELVIRA) used for influenza.

Core C:
During Year 2 period of the project, we will continue with our efforts to perform hit-to-lead and lead optimization chemistry approaches on two chemical series (one main and one back-up) in each of the CHIKV and VEEV projects and on one each chemical series for the SARS, ZIKA, DENV and Influenza projects. Our aim is to generate at least three hit to lead compounds with reasonable ADME and in vivo PK properties that can be evaluated for potency and efficacy in a mouse model as a proof of concept study. These leads will be further optimized with the goal of identifying a pre-clinical candidate(s).

Project 1:
Our efforts in Year 2 will focus on furthering additional hit compounds through the Go/No Go evaluation scheme. Initial tests will involve potency and activity spectrum. Compounds that meet the Go/No Go criteria described previously will undergo passaging for resistance. In addition, we will continue to determine the mechanism of action and resistance of lead compounds, assess the potential to combine compounds by assessing combined potency and cytotoxicity across a dose matrix, and define the effect of resistance to one drug to efficacy of another drug. We will also further evaluate NHC specificity to viral and not host RNA.

Project 2:
Overview: The goal of this project is to identify novel small molecules capable of inhibiting the replication of diverse members of the Alphavirus genus. We have identified a number of hit molecules with antiviral activity that are at different stages of development. Each of the compounds that were described for the upcoming year as active in our program have specific goals associated with each that will influence go/no-go status respectively. This project is the most advanced of all and priority was placed on the most promising leads by the scientific advisory committee.

Project 3:
We will pursue the project as proposed in the original grant in the coming year (see the tasks and timeline table in Project 3 section B.2 and the G.1 report). Briefly, we will continue the following promising directions.
•Perform cocrystal structure-based rational design of dengue capsid inhibitors to achieve improved antiviral spectrum and physicochemical properties.
•Complete hit-to-lead medchem effort on SRI-39748 and SRI-39757 scaffolds (derived from previous screens) to reach go/no-go decision.
•Continue to pursue the Gilead compound for in vivo efficacy in Zika mouse model.
•Test in vivo efficacy for three potential pan-flavivirus nucleoside/nucleotide inhibitors (SRI-43918, EIDD-1931/EIDD-2801, and Remdesivir)

•Complete dengue screen of 200,000 compounds

Project 4:
The overall goal of this project is to identify new therapies that target influenza virus replication. Resistance mutations negate therapeutic benefits of current influenza therapies (M2 and neuraminidase inhibitors). This project will focus on targeting RNA dependent RNA polymerase (RdRp) complex- responsible for many influenza viral functions, including 5´ cap recognition, endonucleolytic cleavage, RNA synthesis, and polyadenylation. Agents that target the distinct functions of the RdRp complex minimize development of resistance since resistance mutations would likely reduce the fidelity of the RdRp. Go/no-go decisions are made based on the following three major goals:
1. Employ a validated HTS primary assay to screen novel drug libraries for antiviral compounds that specifically block influenza virus replication.
2.Characterize the antiviral activity of hit compounds through secondary assays, understand mechanism of action and identify novel RdRp inhibitors
3.Chemical optimization and determination of the in vivo efficacy of lead compounds.

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. OVERALL SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

File(s) uploaded:
Core C.1 Pathak G1.pdf
Core A Admin Whitley G1.pdf
Core B Bostwick G1.pdf
Project 1 Coronavirus - Denison G1.pdf
Core C.2 (b) (3) (A), (b) (6) G1.pdf
Project 2 Alphavirus - Streblow G1.pdf
Project 3 Flavivirus - Shi G1.pdf
Project 4 Influenza - Whitley G1.pdf
G1 CETR Overall Whitley Final.pdf

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Are there personnel on this project who are newly involved in the design or conduct of human subjects research?

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Does this project involve vertebrate animals?

Yes

G.8 PROJECT/PERFORMANCE SITES

Organization Name:	DUNS	Congressional District	Address
Primary: UNIVERSITY OF ALABAMA AT BIRMINGHAM	063690705	AL-007	(b) (6), (b) (3) (A)
UNIVERSITY OF ALABAMA AT BIRMINGHAM	063690705		
UNIVERSITY OF ALABAMA AT BIRMINGHAM	063690705	AL-007	
Oregon Health & Science	096997515	OR-003	

(b) (6), (b) (3) (A)

University		
Vanderbilt University	004413456	TN-005
THe University of North Carolina at Chapel Hill	608195277	NC-004
University of Colorado at Denver	041096314	CO-006
Southern Research Institute	006900526	AL-006
Washington University	068552207	MO-001
Emory University	066469933	GA-05
University of Texas Medical Branch at Galveston	800771149	TX-014

G.9 FOREIGN COMPONENT

No foreign component

G.10 ESTIMATED UNOBLIGATED BALANCE

G.10.a Is it anticipated that an estimated unobligated balance (including prior year carryover) will be greater than 25% of the current year's total approved budget?

No

G.11 PROGRAM INCOME

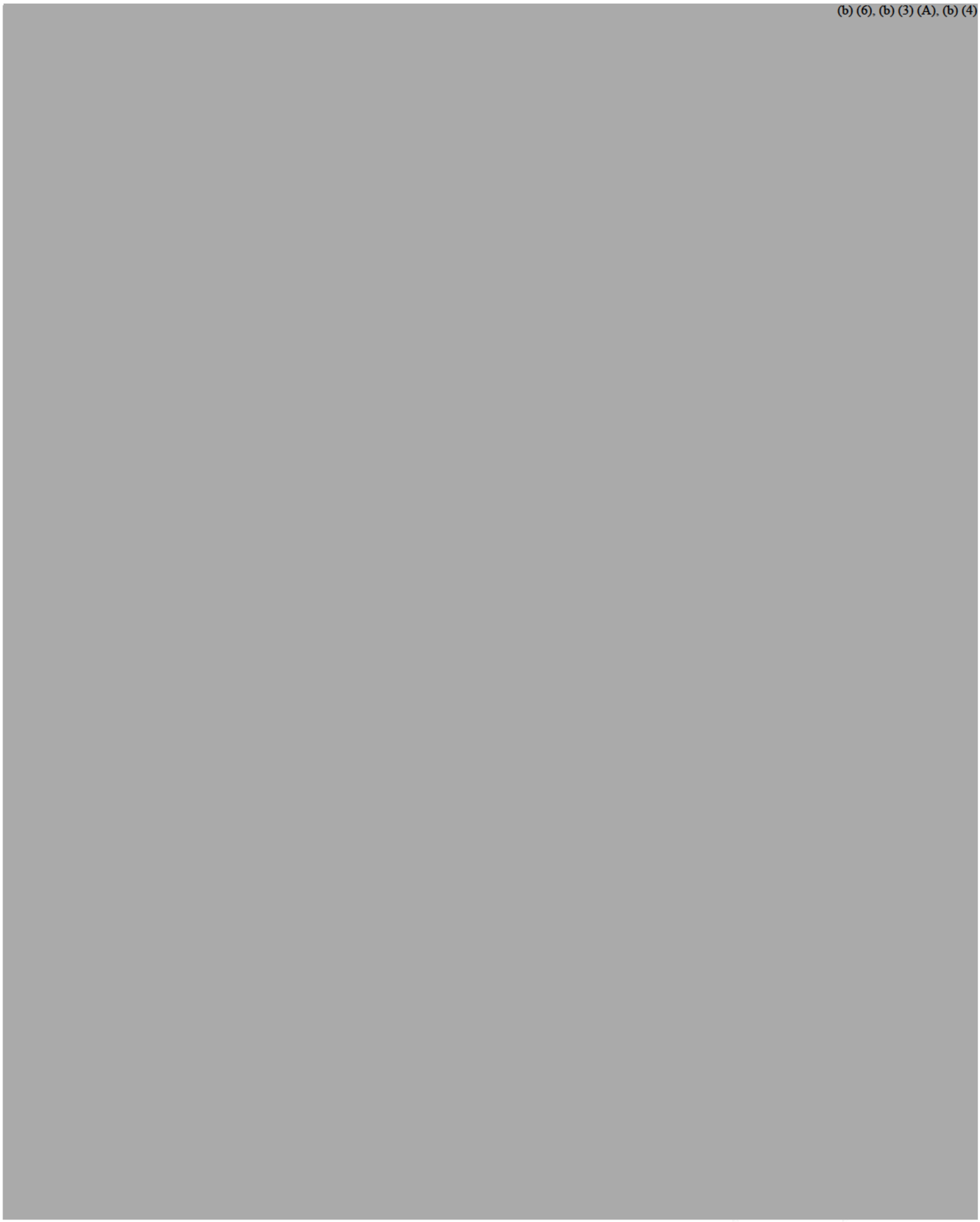
Is program income anticipated during the next budget period?

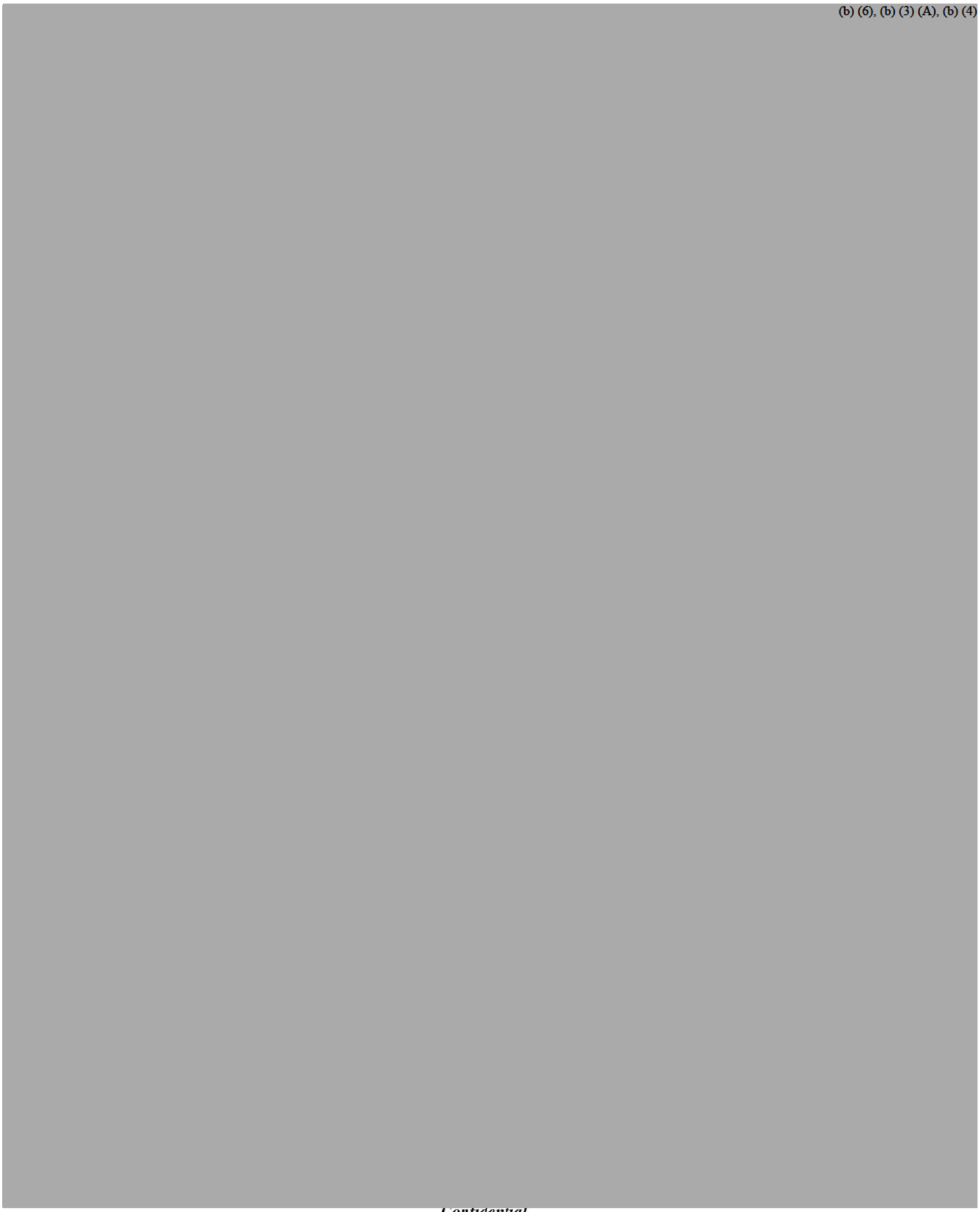
No

G.12 F&A COSTS

Not Applicable

(b) (6), (b) (3) (A), (b) (4)




(b) (6), (b) (3) (A), (b) (4)

(b) (6), (b) (3) (A), (b) (4)


(b) (6), (b) (3) (A), (b) (4)

Core A: Admin Core - Whitley
Section G.1

The Admin Core has served all Projects and Cores by the following activities:

- Coordinated preparation and execution year 1 of renewal grant subaward amendments for all sites including new ones not on the previous award.
- Processed payments to sites following submission of invoices.
- Planned and hosted the sixth annual AD3C meeting (first for award renewal) in which all personnel met in person, thereby facilitating further interactions between projects and cores.
- Provided feedback from the Scientific Advisory Committee reviews to the Projects and Cores.

Core B: Assay Core - Bostwick
Section G.1

Project 1. SARS Corona Virus

Aim 1. Twenty-six (26) synthesized compounds were tested at ten concentrations in an assay measuring nanoluc luciferase activity as an index of virus titer using a recombinant SARS Nanoluc reporter virus produced in the (b) (6), (b) (3). IC_{50} values calculated from this data were used to support SAR for hit-to-lead and lead optimization chemistry efforts

Project 2. Alpha Viruses

Chickungunya virus

Aim 1: Two hundred and twenty-one (221) synthesized compounds were tested in an assay measuring reporter luminescence as an index of virus titer using a recombinant CHIKV Nanoluc virus produced in the Heise lab. IC_{50} values were calculated from the luminescent signal obtained in virus infected THF cells treated at 10 concentrations of test compound as an index of potency. Thirty-two (32) of these compounds were further tested in the VTR assay. Media taken from wells treated with 0, 1 and 10 µM test compounds were serially diluted over 8 logs and used to infect fresh cells. The nanoluc reporter signal in these wells was measured to determine the TCID50 of these diluted samples from which the number of infectious virus particles in the original sample calculated. The maximal fold reduction in the log of the number of infectious virus particles was used as an index of compound efficacy. These combined data were used to drive SAR for hit-to-lead and lead optimization chemistry efforts.

Venezuelan Equine Encephalitis virus

Aim 1: Two hundred and twenty-one (221) synthesized compounds were tested at ten concentrations in a CPE assay and IC_{50} values were calculated as an index of compound potency. Six (6) of these compounds were further tested in the VTR assay. Media taken from wells treated with 0, 1 and 10 uM compound were serially diluted over 8 logs and used to infect fresh cells. CPE was measured to determine the TCID50 of these diluted samples from which the number of infectious virus particles in the original sample was calculated. The maximal fold reduction in the log of the number of infectious virus particles was used as an index of compound efficacy. These combined data were used to drive SAR for hit-to-lead and lead optimization chemistry efforts.

Project 3. Flaviviruses

Dengue virus

Aim 1: Sixty-nine (69) compounds were tested at ten concentrations in an immunofluorescence assay measuring viral protein expression in HEK293 host cells. IC_{50} values were calculated from this data as an index of compound potency. None of these compounds were submitted for testing in the VTR assay. These data were used to drive SAR for hit-to-lead and lead optimization chemistry efforts.

Aim 2: Currently, the project has only one lead compound derived from a previous HTS campaign that employed a CPE assay to measure anti-viral effects. In order to identify

additional chemical scaffolds for this project, we are conducting another HTS campaign using the immunofluorescence assay used to drive SAR. We are screening 200,000 compounds from a ChemDiv library because these compounds are structurally different from the Enamine and ChemBridge collections screened previously. The screen is ongoing at the time of this writing but will be completed by the end of year one.

Zika Virus

Aim 1: One hundred and twenty (120) compounds have been tested in an immunofluorescence assay measuring Zika viral protein expression in Vero CCL81 host cells. IC_{50} values were calculated from this data as an index of compound potency. None of these compounds were submitted for testing in the VTR assay. These data were used to drive SAR for hit-to-lead and lead optimization chemistry efforts.

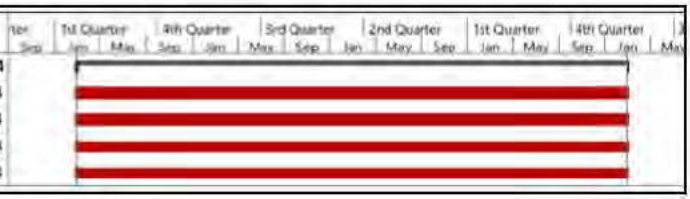
Project 4. Influenza A viruses

Aim 1: One hundred and fifty-four (154) synthesized compounds were tested at 10 concentrations in an immunofluorescence assays to measure anti-viral effects against H3N2 (Udorn) and H1N1 (Wisconsin). IC_{50} values were calculated to determine potency. None of these compounds were submitted for testing in the VTR assay.

Aim 2: To identify additional hits for this project, we used an immunofluorescence assay measuring surface expression of M2 protein in H3N2 infected A549 host cells to screen a 200K library from ChemDiv. Compound structures in this collection are distinct from those previously screened. This screen identified 140 non-cytotoxic (CC_{50} > 30 µM) compounds with IC_{50} < 30 µM.

Timeline

Task Name	Start	Finish
Assay Core	**Sun 3/1/20**	**Thu 2/29/24**
Project 1 SAR Supporting Assays	Sun 3/1/20	Thu 2/29/24
Project 2 SAR Supporting Assays	Sun 3/1/20	Thu 2/29/24
Project 3 SAR Supporting Assays	Sun 3/1/20	Thu 2/29/24
Project 4 SAR Supporting Assays	Sun 3/1/20	Thu 2/29/24

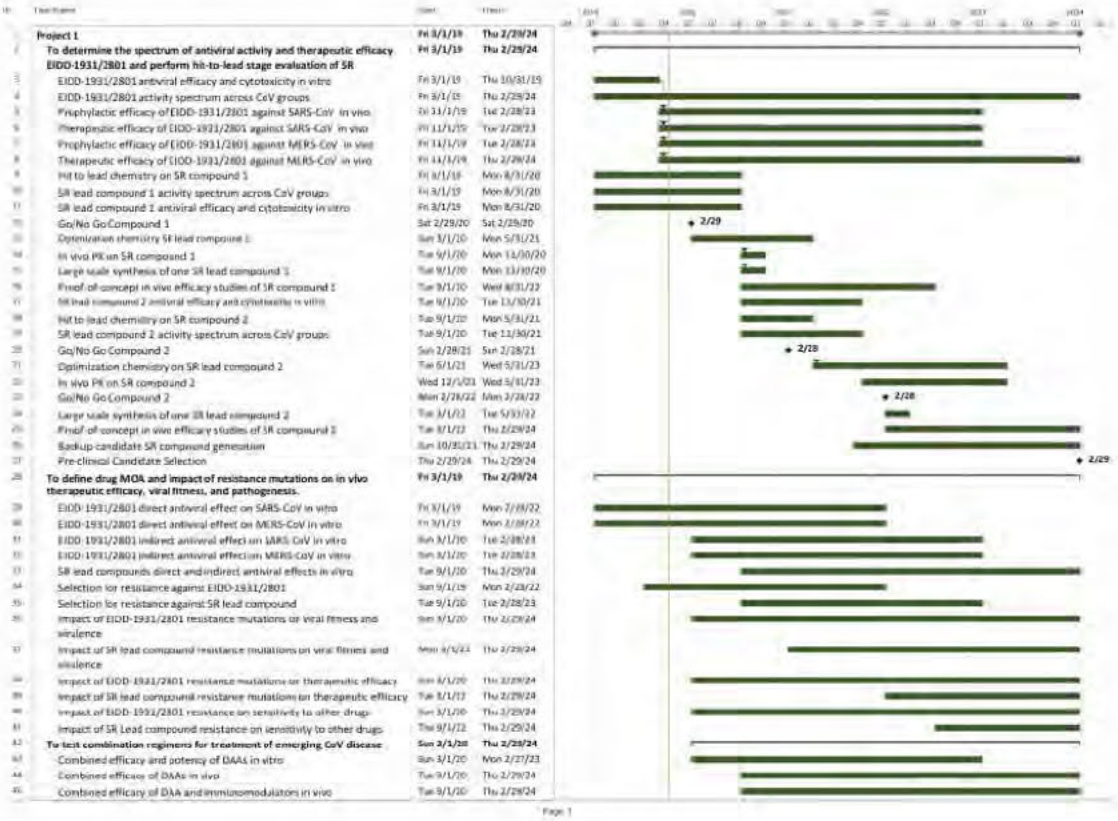


Project 1: Coronavirus – Denison
Section G.1 RPPR

Significant changes in the specific aims:
None to report.

Significance of the work:
In this Project we will use extensive small molecule libraries and a sensitive high-throughput in vitro screening assay to identify inhibitors of SARS-CoV replication fidelity and RNA capping that will lead to profound in vivo attenuation, and potentially represent broadly-efficacious inhibitors of endemic and emerging CoVs.

Product development milestones:
Overall project progress and product development is tracked via the following timeline; the end of year 1 is noted by an orange line:



1	**Project 1**	**Fri 3/1/19**	**Thu 2/29/24**
2	**To determine the spectrum of antiviral activity and therapeutic efficacy EIDD-1931/2801 and perform hit-to-lead stage evaluation of SR**	**Fri 3/1/19**	**Thu 2/29/24**
3	EIDD-1931/2801 antiviral efficacy and cytotoxicity in vitro	Fri 3/1/19	Thu 10/31/19
4	EIDD-1931/2801 activity spectrum across CoV groups	Fri 3/1/19	Thu 2/29/24
5	Prophylactic efficacy of EIDD-1931/2801 against SARS-CoV in vivo	Fri 11/1/19	Tue 2/28/23
6	Therapeutic efficacy of EIDD-1931/2801 against SARS-CoV in vivo	Fri 11/1/19	Tue 2/28/23
7	Prophylactic efficacy of EIDD-1931/2801 against MERS-CoV in vivo	Fri 11/1/19	Tue 2/28/23
8	Therapeutic efficacy of EIDD-1931/2801 against MERS-CoV in vivo	Fri 11/1/19	Thu 2/29/24
9	Hit to lead chemistry on SR compound 1	Fri 3/1/19	Mon 8/31/20
10	SR lead compound 1 activity spectrum across CoV groups	Fri 3/1/19	Mon 8/31/20
11	SR lead compound 1 antiviral efficacy and cytotoxicity in vitro	Fri 3/1/19	Mon 8/31/20
12	Go/No Go Compound 1	Sat 2/29/20	Sat 2/29/20
13	Optimization chemistry SR lead compound 1	Sun 3/1/20	Mon 5/31/21
14	In vivo PK on SR compound 1	Tue 9/1/20	Mon 11/30/20
15	Large scale synthesis of one SR lead compound 1	Tue 9/1/20	Mon 11/30/20
16	Proof-of-concept in vivo efficacy studies of SR compound 1	Tue 9/1/20	Wed 8/31/22
17	SR lead compound 2 antiviral efficacy and cytotoxicity in vitro	Tue 9/1/20	Tue 11/30/21
18	Hit to lead chemistry on SR compound 2	Tue 9/1/20	Mon 5/31/21
19	SR lead compound 2 activity spectrum across CoV groups	Tue 9/1/20	Tue 11/30/21
20	Go/No Go Compound 2	Sun 2/28/21	Sun 2/28/21
21	Optimization chemistry on SR lead compound 2	Tue 6/1/21	Wed 5/31/23
22	In vivo PK on SR compound 2	Wed 12/1/21	Wed 5/31/23
23	Go/No Go Compound 2	Mon 2/28/22	Mon 2/28/22
24	Large scale synthesis of one SR lead compound 2	Tue 3/1/22	Tue 5/31/22
25	Proof-of-concept in vivo efficacy studies of SR compound 2	Tue 3/1/22	Thu 2/29/24
26	Backup candidate SR compound generation	Sun 10/31/21	Thu 2/29/24
27	Pre-clinical Candidate Selection	Thu 2/29/24	Thu 2/29/24
28	**To define drug MOA and impact of resistance mutations on in vivo therapeutic efficacy, viral fitness, and pathogenesis.**	**Fri 3/1/19**	**Thu 2/29/24**
29	EIDD-1931/2801 direct antiviral effect on SARS-CoV in vitro	Fri 3/1/19	Mon 7/18/22
30	EIDD-1931/2801 direct antiviral effect on MERS-CoV in vitro	Fri 3/1/19	Mon 7/18/22
31	EIDD-1931/2801 indirect antiviral effect on SARS-CoV in vitro	Sun 3/1/20	Tue 2/28/23
32	EIDD-1931/2801 indirect antiviral effect on MERS-CoV in vitro	Sun 3/1/20	Tue 2/28/23
33	SR lead compounds direct and indirect antiviral effects in vitro	Tue 9/1/20	Thu 2/29/24
34	Selection for resistance against EIDD-1931/2801	Sun 9/1/19	Mon 2/28/22
35	Selection for resistance against SR lead compound	Tue 9/1/20	Tue 2/28/23
36	Impact of EIDD-1931/2801 resistance mutations on viral fitness and virulence	Sun 3/1/20	Thu 2/29/24
37	Impact of SR lead compound resistance mutations on viral fitness and virulence	Mon 8/1/22	Thu 2/29/24
38	Impact of EIDD-1931/2801 resistance mutations on therapeutic efficacy	Sun 3/1/20	Thu 2/29/24
39	Impact of SR lead compound resistance mutations on therapeutic efficacy	Tue 3/1/22	Thu 2/29/24
40	Impact of EIDD-1931/2801 resistance on sensitivity to other drugs	Sun 3/1/20	Thu 2/29/24
41	Impact of SR Lead compound resistance on sensitivity to other drugs	Tue 9/1/22	Thu 2/29/24
42	**To test combination regimens for treatment of emerging CoV disease**	**Sun 3/1/20**	**Thu 2/29/24**
43	Combined efficacy and potency of DAAs in vitro	Sun 3/1/20	Mon 2/27/23
44	Combined efficacy of DAAs in vivo	Tue 9/1/20	Thu 2/29/24
45	Combined efficacy of DAA and immunomodulators in vivo	Tue 9/1/20	Thu 2/29/24

Significant project-generated resources:
None to report.

CETR RPPR Section G.1 for Core C.2 - (b) (3) (A), (b) (6)

Core C.2: Medicinal Chemistry and Lead Development Core (MCLDC) at Emory Institute for Drug Development

The Emory Institute for Drug Development (EIDD) is an important component of the Antiviral Drug Discovery and Development Center (AD3C) U19 grant. EIDD has a compound library that consist of ribonucleoside and ribonucleotide analogues specifically designed as competitive, alternative substrate inhibitors of virally encoded RNA-dependent RNA polymerases (RdRp). These molecules provide a unique opportunity to study broadly active antiviral compounds. As such, in a similar manner to their natural ribonucleoside counterparts, these ribonucleoside analogues must initially be recognized and anabolized by cellular kinases in three distinct steps to their activated 5'-triphosphate form to inhibit viral RdRps. Once incorporated into the viral RNA, these 5'-triphosphate ribonucleotide analogs inhibit nascent chain RNA synthesis either by obligate termination or by decreasing the efficiency of subsequent ribonucleoside additions to the chain. Alternatively, ribonucleoside analog 5'-triphosphates can be incorporated into nascent chain RNA, rendering it non-functional by perturbing the viral RNA secondary structure.

The broadly active ribonucleoside analog, EIDD-2749 (4'-fluorouridine), was confirmed to have antiviral activity (EC_{50} = 3.47 μM) in a human airway epithelial model of MERS infection in **Project 1 of AD3C**. The EIDD will continue to supply material to support in vitro screening, molecular virology, and in vivo efficacy models to further characterize EIDD-2749 against coronaviruses.

During the study of these drugs, it has been learned that, in order to be utilized by the virally encoded RdRp as competitive alternative substrate inhibitors, ribonucleoside analogs must be converted to their 5'-triphosphates. However, host kinases often fail to recognize the ribonucleoside analogs as substrates (particularly for the formation of the 5'-mono and 5'-diphosphates), and as a consequence no activity is observed in cell-based models of infection. Therefore, many compounds that would be highly effective agents if they were phosphorylated are considered inactive. To overcome these anabolic blocks in cells and generate adequate levels of ribonucleotide analog 5'-triphosphate for RdRp inhibition, a prodrug strategy was developed using 5'-mono and diphosphate analogs as means to by-pass the initial anabolic steps. The development of 2'-C-methyl-2-thiouridine (EIDD-1850) as an antiviral agent against HCV infection illustrates the prodrug strategy in identifying a competitive inhibitor of HCV encoded RdRp. Tested against the HCV-1b replicon assay in human hepatoma Huh-7 cells, ribonucleoside analog EIDD-1850 showed no observable inhibition (EC_{50} > 200 uM) whereas the EIDD-1850-5'-phosphoramidate prodrug (EIDD-2023) exhibited submicromolar inhibition (EC_{50} = 0.03 uM) in the HCV-1b replicon system. Designed as a liver targeting prodrug, EIDD-2023 phosphoramidate is rapidly metabolized in human hepatocytes via a mechanism initiated by cellular carboxypeptidase and followed by phosphoramidase (Hint-1) release of the 5'-monophosphate nucleoside that is anabolized to its active 5'-triphosphate. Another prodrug strategy that is utilized to establish

cellular antiviral activity with ribonucleosides that are poorly anabolized is the bis(pivaloyloxymethyl)phosphate prodrug. In a manner similar to phosphoramidate prodrugs, bis(pivaloyloxymethyl)phosphate (POM) prodrug unmasking is initiated by ester cleavage by cellular carboxyesterases followed by rapid hydrolysis to release the 5'-monophosphate anabolite.

These prodrug strategies were used to generate a series of nine 2'-substituted pyrimidine ribonucleotide analogs as their 5'-monophosphate prodrugs. These compounds were submitted to **Project 3 of the AD3C** to confirm and elaborate on their anti-flavivirus activity. After establishing antiviral activity in cell models of flavivirus infections, compounds showing broad activity will be advanced into in vitro stability assays as well as cellular assays to establish anabolic profiles of 5'-triphosphate formation prior to conducting in vivo pharmacokinetic/pharmacodynamic studies. These studies are now being undertaken by (b) (3) (A), (b) (6) **in Project 3**.

An example of a broadly active lead is EIDD-2801 that is a broadly active ribonucleoside analog in development to treat seasonal influenza and alphavirus infections. 28-day GLP toxicology studies and rats and dogs have been completed, which established a NOAEL of 6mg/kg. **Project 4 of AD3C** is evaluating analogs of EIDD 2801. The EIDD will continue to supply EIDD-2801 to support in vivo efficacy studies against flaviviruses.

EIDD-2838, the 5'-cyclosal monophosphate prodrug of EIDD-2749, is a broadly active ribonucleotide analog that was found to inhibit the cap-snatching activity of the influenza virus (EC_{50} = 2-4 μM). A series of 15 analogs were prepared to establish structure activity relationships in the cap-snatching assay. Additional analogs will be prepared based on the results from these 15 compounds to further optimize activity and stability prior to moving into in vivo efficacy models of influenza infection.

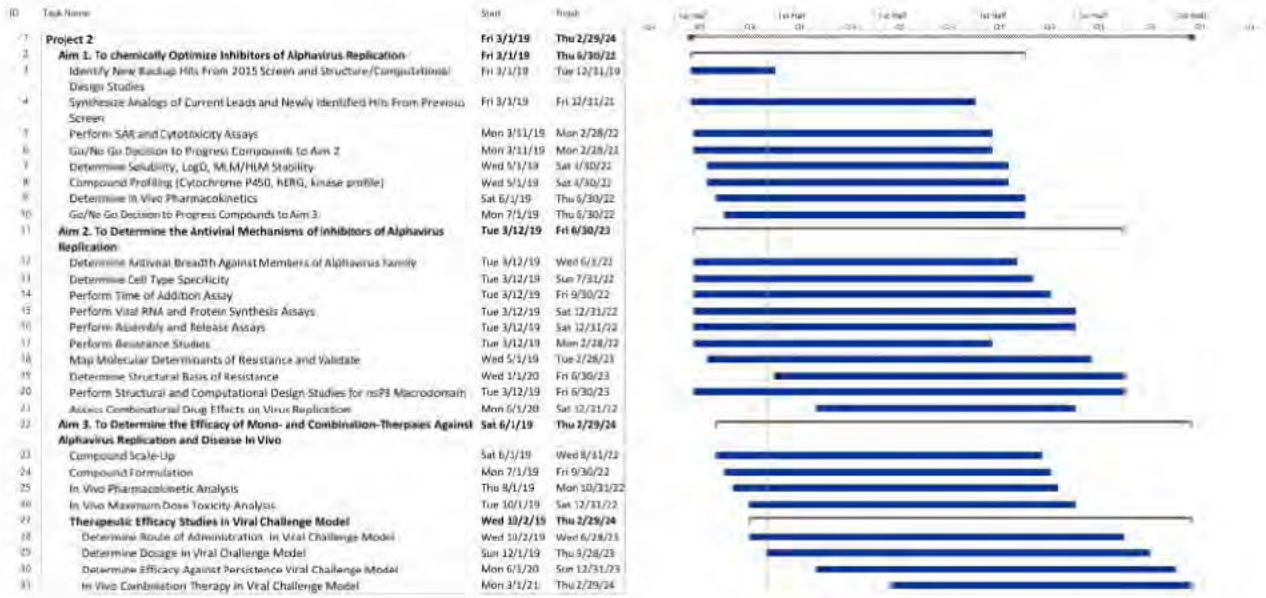
In summary, EIDD is contributing leads to all AD3C Projects in order to define the spectrum of activity and mechanism of action. In addition, important proof of principle data will be obtained in animal models that will inform preclinical toxicology studies.

Project 2: Alphavirus – Streblow
Section G.1 RPPR

Significant changes in the specific aims:
None to report.

Significance of the work:
Our Program aims to develop novel antiviral agents to emerging human viral pathogens. This project will develop broad-spectrum nucleoside/nucleotide inhibitors against Alphaviruses with a focus on Chikungunya virus and Venezuelan Equine Encephalitis virus, both of which are human pathogens that cause severe disease and are associated with mortality and with no currently FDA approved vaccine or therapeutics for treatment.

Product development milestones:
Overall project progress and product development is tracked via the following timeline; the end of year 1 is noted by an orange line:



ID	Task Name	Start	Finish
1	**Project 2**	**Fri 3/1/19**	**Thu 2/29/24**
2	**Aim 1. To chemically Optimize Inhibitors of Alphavirus Replication**	**Fri 3/1/19**	**Thu 6/30/22**
3	Identify New Backup Hits From 2015 Screen and Structure/Computational Design Studies	Fri 3/1/19	Tue 12/31/19
4	Synthesize Analogs of Current Leads and Newly Identified Hits From Previous Screen	Fri 3/1/19	Fri 12/31/21
5	Perform SAR and Cytotoxicity Assays	Mon 3/11/19	Mon 2/28/22
6	Go/No Go Decision to Progress Compounds to Aim 2	Mon 3/11/19	Mon 2/28/21
7	Determine Solubility, LogD, MLM/HLM Stability	Wed 5/1/19	Sat 4/30/22
8	Compound Profiling (Cytochrome P450, hERG, kinase profile)	Wed 5/1/19	Sat 4/30/22
9	Determine In Vivo Pharmacokinetics	Sat 6/1/19	Thu 6/30/22
10	Go/No Go Decision to Progress Compounds to Aim 3	Mon 7/1/19	Thu 6/30/22
11	**Aim 2. To Determine the Antiviral Mechanisms of Inhibitors of Alphavirus Replication**	**Tue 3/12/19**	**Fri 6/30/23**
12	Determine Antiviral Breadth Against Members of Alphavirus Family	Tue 3/12/19	Wed 6/1/22
13	Determine Cell Type Specificity	Tue 3/12/19	Sun 7/31/22
14	Perform Time of Addition Assay	Tue 3/12/19	Fri 9/30/22
15	Perform Viral RNA and Protein Synthesis Assays	Tue 3/12/19	Sat 12/31/22
16	Perform Assembly and Release Assays	Tue 3/12/19	Sat 12/31/22
17	Perform Resistance Studies	Tue 3/12/19	Mon 2/28/22
18	Map Molecular Determinants of Resistance and Validate	Wed 5/1/19	Tue 2/28/23
19	Determine Structural Basis of Resistance	Wed 1/1/20	Fri 6/30/23
20	Perform Structural and Computational Design Studies for nsP3 Macrodomain	Tue 3/12/19	Fri 6/30/23
21	Assess Combinatorial Drug Effects on Virus Replication	Mon 6/1/20	Sat 12/31/22
22	**Aim 3. To Determine the Efficacy of Mono- and Combination-Therpaies Against Alphavirus Replication and Disease In Vivo**	**Sat 6/1/19**	**Thu 2/29/24**
23	Compound Scale-Up	Sat 6/1/19	Wed 8/31/22
24	Compound Formulation	Mon 7/1/19	Fri 9/30/22
25	In Vivo Pharmacokinetic Analysis	Thu 8/1/19	Mon 10/31/22
40	In Vivo Maximum Dose Toxicity Analysis	Tue 10/1/19	Sat 12/31/22
27	**Therapeutic Efficacy Studies in Viral Challenge Model**	**Wed 10/2/19**	**Thu 2/29/24**
28	Determine Route of Administration in Viral Challenge Model	Wed 10/2/19	Wed 6/28/23
29	Determine Dosage in Viral Challenge Model	Sun 12/1/19	Thu 9/28/23
30	Determine Efficacy Against Persistence Viral Challenge Model	Mon 6/1/20	Sun 12/31/23
31	In Vivo Combination Therapy in Viral Challenge Model	Mon 3/1/21	Thu 2/29/24

Significant project-generated resources:
None to report.

Project 3: Flavivirus – Shi
Section G.1 RPPR

Significant changes in the specific aims:
None to report.

Significance of the work:
The flaviviruses are associated with significant morbidity, mortality, and economic burden throughout world. Nevertheless, no specific anti-viral therapies for disease associated with these viruses are currently available. This project is designed to identify and develop small molecule anti-viral therapeutics against two medically important flaviviruses--dengue virus and West Nile virus. Furthermore, we will emphasize the development of drugs that show activity against multiple flaviviruses, and possibly other virus families as well.

Product development milestones:
Overall project progress and product development is tracked via the following timeline; the end of year 1 is noted by an orange line:

ID	Task Name	Start	Finish
1	**Flavivirus**	**Fri 3/1/19**	**Fri 3/1/24**
2	**Aim 1**	**Fri 3/1/19**	**Fri 9/1/23**
3	Establish SAR for DENV C inhibitor	Fri 3/1/19	Tue 8/31/21
4	Determine co-crystal structures of DENV C and inhibitor	Fri 5/31/19	Mon 2/28/22
5	Hit to lead optimization of flavivirus hits from previous screen	Fri 3/1/19	Tue 8/31/21
6	Pharmacokinetics, bioavailability & biodistribution studies	Fri 3/1/19	Mon 2/28/22
7	Screen new libraries for back-up series	Fri 3/1/19	Sun 2/28/21
8	Compound efficacy testing in ZIKV mouse model	Mon 8/31/20	Thu 8/31/23
9	Identify potent flavivirus compounds with in vivo activity	Fri 9/1/23	Fri 9/1/23
10	**Aim 2**	**Fri 3/1/19**	**Thu 8/31/23**
11	Identify Nuc inhibitors through current Nuc collection	Fri 3/1/19	Sat 2/29/20
12	MedChem prodrug	Mon 3/2/20	Tue 2/28/23
13	Determine EC50, CC50 & Toxicity	Mon 3/2/20	Tue 2/28/23
14	Resistance analysis and antiviral spectrum	Tue 9/1/20	Tue 2/28/23
15	Combination efficacy studies in mouse model	Tue 9/1/20	Thu 8/31/23
16	**Aim 3**	**Mon 3/1/21**	**Fri 3/1/24**
17	Perform combination efficacy in cells	Mon 3/1/21	Tue 2/28/23
18	Perform resistance analysis	Mon 3/1/21	Tue 2/28/23
19	Determine EC50 & CC50	Tue 6/1/21	Tue 2/28/23
20	Determine mouse efficacy	Tue 6/1/21	Thu 8/31/23
21	Compound profiling (Cytochrome P450, hERG, kinase profile)	Thu 9/1/22	Tue 2/28/23
22	Determine NHP efficacy	Wed 3/1/23	Thu 2/29/24
23	Selection of preclinical candidate	Fri 3/1/24	Fri 3/1/24



Significant project-generated resources:
None to report.

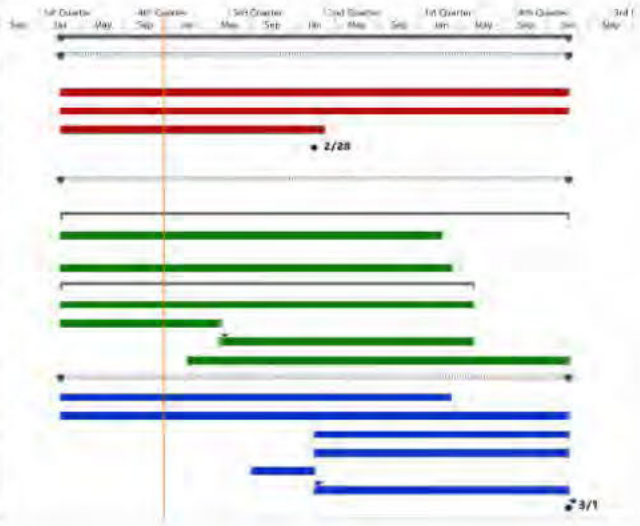
Project 4: Influenza – Whitley
Section G.1 RPPR

Significant changes in the specific aims:
None to report.

Significance of the work:
Influenza A viruses continue to emerge from the aquatic avian reservoir and cause seasonal epidemics and infrequent pandemics. Recent experimental evidence by our group and others support the development of novel antivirals targeting the influenza polymerase function. The discovery of molecules that inhibit influenza virus RNA replication is essential to complement the existing drug arsenal, which is proving less effective due to the increasing incidence of mutational resistance. Targeting RNA dependent RNA polymerase (RdRp) complex: Responsible for many viral functions, including 5´ cap recognition, endonucleolytic cleavage, RNA synthesis, and polyadenylation. Agents that target the distinct functions of the RdRp complex minimize development of resistance since resistance mutations would likely reduce the fidelity of the RdRp.

Product development milestones:
Overall project progress and product development is tracked via the following timeline; the end of year 1 is noted by an orange line:

ID	Task Name	Start	Finish
1	**Project 4**	**Sun 3/1/20**	**Fri 3/1/24**
2	**Aim 1. Identify Potent Influenza Compounds Targeting RdRp Complex Functions**	**Sun 3/1/20**	**Thu 2/29/24**
3	Hit to Lead Optimization of Newly Identified Hits	Sun 3/1/20	Thu 2/29/24
4	Screening Compound for Anti-Influenza Activities	Sun 3/1/20	Thu 2/29/24
5	Synthesize Additional Nucleoside Analogs	Sun 3/1/20	Thu 3/31/22
6	Identify Potent Influenza Compounds Targeting RdRp Complex Functions	Mon 2/28/22	Mon 2/28/22
7	**Aim 2. Define Mechanism of Action and Antiviral Spectrum**	**Sun 3/1/20**	**Thu 2/29/24**
8	**Define Spectrum of Antiviral Activity**	**Mon 3/2/20**	**Wed 2/28/24**
9	Assessment of activity in H1N1, H3N2, B strains (B/Yamagata and B/Victoria lineage) and HPAI	Sun 3/1/20	Tue 2/28/23
10	Confirm RdRp Function Inhibition	Sun 3/1/20	Fri 3/31/23
11	**Resistance Studies**	**Sun 3/1/20**	**Wed 5/31/23**
12	Map Molecular Determinants of Resistance	Sun 3/1/20	Wed 5/31/23
13	Structure Based Determination of Resistance	Sun 3/1/20	Wed 6/2/21
14	Target Identification and Optimization	Thu 6/3/21	Wed 5/31/23
15	Combination Efficacy Studies In Vitro	Mon 3/1/21	Thu 2/29/24
16	**Aim 3. In Vivo Evaluation & Bioavailability Studies**	**Sun 3/1/20**	**Fri 3/1/24**
17	Pharmacokinetics, Bioavailability & Biodistribution Studies	Sun 3/1/20	Wed 3/29/23
18	Efficacy Testing in H1N1 Mouse Model	Sun 3/1/20	Thu 2/29/24
19	Efficacy Testing in H5N1 Mouse Model	Tue 3/1/22	Thu 2/29/24
20	Combination Efficacy Testing in H1N1 Mouse Model	Tue 3/1/22	Thu 2/29/24
21	Compound Profiling (Cytochrome P450, hERG, kinase profile)	Wed 9/1/21	Mon 2/28/22
22	Efficacy Testing in H1N1 Ferret Model	Tue 3/1/22	Thu 2/29/24
23	Selection of Preclinical Candidate	Fri 3/1/24	Fri 3/1/24



Significant project-generated resources:
None to report.

G.1 CETR Overall – Whitley

1. Product Development

Not Applicable

2. Biocontainment/Security:

Project 1:

1. Project Title: The Development of Antivirals for Coronavirus Replication
2. Project Leader: Denison, MR (Vanderbilt),
3. Collaborator(s): (b) (6), (b) (3), RS (UNC)
4. BSL Laboratory Employed: BSL3 (Vanderbilt) BSL3/ABSL3 (UNC)
5. Pathogen(s) Evaluated: Both Vanderbilt and UNC: SARS-CoV, MERS-CoV. UNC only: HKU3, HKU5, SH014, SADS-CoV Both Vanderbilt and UNC Select Agent Certified

Project 2:

1. Project title: Novel therapeutic strategies targeting re-emerging alphaviruses
2. Project Leader: Daniel Streblow (OHSU)
3. Project Collaborators: (b) (6), (b) (3) (A) (UNC), (b) (6), (b) (3) (A) (OHSU) and (b) (6), (b) (3) (A) (U Colorado Denver)
4. Labs at the following sites were used:
    a. Vaccine & Gene Therapy Institute/Oregon Health & Science University.
        i. BSL-3/VGTI rm2215A, Small Animal ABSL-3/VGTI
        ii. Pathogen: Chikungunya Virus
    b. University of North Carolina-Chapel Hill.
        i. BSL-3, Room (b) (6), (b) (3), Small Animal ABSL-3, Rooms (b) (6), (b) (3) (A), Tissue culture BSL-3.
        ii. Pathogen: Chikungunya virus, Venezuelan equine encephalitis virus
    c. University of Colorado-Denver.
        i. (b) (6), (b) (3) (A) BSL3 Facility
        ii. Pathogen: Chikungunya Virus

Core B utilized the BSL3 facility at Southern Research for the following projects:

Project number: 1
Project Title: Inhibitors of Coronavirus
Project Leaders: Mark Denison, (b) (6), (b) (3) (A)
Collaborators:
BSL Laboratory Employed: Southern Research Institute (for HTS activities)
Pathogens Evaluated: SARS Toronto-2 and SARS Urbani/Nanoluc Clone

3. Follow-on Funding

None to report

Composite Application Budget Summary

Categories	Budget Period
Salary, Wages and Fringe Benefits	197,341
Equipment	0
Travel	20,288
Participant/Trainee Support Costs	0
Human Fetal Tissue Costs*	
Other Direct Costs (excluding Consortium)	63,389
Consortium Costs	7,082,681
Direct Costs	7,363,699
Indirect Costs	136,294
Total Direct and Indirect Costs	7,499,993

*Human Fetal Tissue Costs, if present, are included in the budget summary for information only; the values have not been subtracted from "Other Direct Costs (excluding Consortium)"

*This application includes at least one component led by an organization that has a DUNS different than the Applicant Organization. The indirect cost calculation for the applicant organization may not include all allowed Indirect Costs for the first $25K of requested consortium costs and, therefore, may appear less than expected. No action is required from the applicant; NIH will make any appropriate corrections to the budget calculations administratively. The application review will not be affected.

Component Budget Summary

Components	Categories	Budget Period
6072-001 (Admin Core)	Salary, Wages and Fringe Benefits	91,474
	Equipment	0
	Travel	18,788
	Participant/Trainee Support Costs	0
	Human Fetal Tissue Costs	
	Other Direct Costs (excluding Consortium)	31,480
	Consortium Costs	0
	Direct Costs	141,742
	Indirect Costs	68,745
TOTALS	Total Direct and Indirect Costs	210,487
6073-001 (Core)	Salary, Wages and Fringe Benefits	206,475
	Equipment	0
	Travel	5,150
	Participant/Trainee Support Costs	0
	Human Fetal Tissue Costs	
	Other Direct Costs (excluding Consortium)	265,498
	Consortium Costs	0
	Direct Costs	477,123
	Indirect Costs	405,450
TOTALS	Total Direct and Indirect Costs	882,573

6075-002 (Core)	Salary, Wages and Fringe Benefits	116,152
	Equipment	0
	Travel	0
	Participant/Trainee Support Costs	0
	Human Fetal Tissue Costs	
	Other Direct Costs (excluding Consortium)	63,848
	Consortium Costs	0
	Direct Costs	180,000
	Indirect Costs	141,300
TOTALS	Total Direct and Indirect Costs	321,300
6074-003 (Core)	Salary, Wages and Fringe Benefits	730,904
	Equipment	0
	Travel	5,150
	Participant/Trainee Support Costs	0
	Human Fetal Tissue Costs	
	Other Direct Costs (excluding Consortium)	216,977
	Consortium Costs	0
	Direct Costs	953,031
	Indirect Costs	1,287,330
TOTALS	Total Direct and Indirect Costs	2,240,361
6301-001 (Project)	Salary, Wages and Fringe Benefits	213,076
	Equipment	0
	Travel	7,500

	Participant/Trainee Support Costs	3,450
	Human Fetal Tissue Costs	
	Other Direct Costs (excluding Consortium)	140,937
	Consortium Costs	0
	Direct Costs	364,963
	Indirect Costs	200,640
TOTALS	Total Direct and Indirect Costs	565,603
6303-002 (Project)	Salary, Wages and Fringe Benefits	101,094
	Equipment	0
	Travel	4,000
	Participant/Trainee Support Costs	3,000
	Human Fetal Tissue Costs	
	Other Direct Costs (excluding Consortium)	58,977
	Consortium Costs	0
	Direct Costs	167,071
	Indirect Costs	91,059
TOTALS	Total Direct and Indirect Costs	258,130
6311-003 (Project)	Salary, Wages and Fringe Benefits	126,176
	Equipment	0
	Travel	1,500
	Participant/Trainee Support Costs	0
	Human Fetal Tissue Costs	
	Other Direct Costs (excluding Consortium)	32,582

	Consortium Costs	0
	Direct Costs	160,258
	Indirect Costs	86,539
TOTALS	Total Direct and Indirect Costs	246,797
6076-004 (Project)	Salary, Wages and Fringe Benefits	192,073
	Equipment	0
	Travel	6,000
	Participant/Trainee Support Costs	0
	Human Fetal Tissue Costs	
	Other Direct Costs (excluding Consortium)	92,288
	Consortium Costs	0
	Direct Costs	290,361
	Indirect Costs	168,409
TOTALS	Total Direct and Indirect Costs	458,770
6077-005 (Project)	Salary, Wages and Fringe Benefits	269,875
	Equipment	0
	Travel	2,000
	Participant/Trainee Support Costs	0
	Human Fetal Tissue Costs	
	Other Direct Costs (excluding Consortium)	61,324
	Consortium Costs	0
	Direct Costs	333,199
	Indirect Costs	179,928

TOTALS	Total Direct and Indirect Costs	513,127
6309-006 (Project)	Salary, Wages and Fringe Benefits	100,356
	Equipment	0
	Travel	2,499
	Participant/Trainee Support Costs	0
	Human Fetal Tissue Costs	
	Other Direct Costs (excluding Consortium)	128,865
	Consortium Costs	0
	Direct Costs	231,720
	Indirect Costs	121,653
TOTALS	Total Direct and Indirect Costs	353,373
6079-007 (Project)	Salary, Wages and Fringe Benefits	105,867
	Equipment	0
	Travel	1,500
	Participant/Trainee Support Costs	0
	Human Fetal Tissue Costs	
	Other Direct Costs (excluding Consortium)	31,909
	Consortium Costs	0
	Direct Costs	139,276
	Indirect Costs	67,549
TOTALS	Total Direct and Indirect Costs	206,825
6078-008 (Project)	Salary, Wages and Fringe Benefits	173,298
	Equipment	0

	Travel	1,500
	Participant/Trainee Support Costs	0
	Human Fetal Tissue Costs	
	Other Direct Costs (excluding Consortium)	28,345
	Consortium Costs	0
	Direct Costs	203,143
	Indirect Costs	117,823
TOTALS	Total Direct and Indirect Costs	320,966
6310-009 (Project)	Salary, Wages and Fringe Benefits	194,440
	Equipment	0
	Travel	5,238
	Participant/Trainee Support Costs	0
	Human Fetal Tissue Costs	
	Other Direct Costs (excluding Consortium)	117,794
	Consortium Costs	0
	Direct Costs	317,472
	Indirect Costs	355,317
TOTALS	Total Direct and Indirect Costs	672,789
6307-010 (Project)	Salary, Wages and Fringe Benefits	79,299
	Equipment	0
	Travel	3,000
	Participant/Trainee Support Costs	0
	Human Fetal Tissue Costs	

	Other Direct Costs (excluding Consortium)	77,760
	Consortium Costs	0
	Direct Costs	160,059
	Indirect Costs	88,833
TOTALS	Total Direct and Indirect Costs	248,892
TOTALS		**7,499,993**

Categories Budget Summary

Categories	Components	Budget Period
R&R Budget - Senior/Key Person Funds Requested	6072-001 (Admin Core)	60,549
	6073-001 (Core)	48,813
	6075-002 (Core)	4,938
	6074-003 (Core)	78,182
	6301-001 (Project)	125,961
	6303-002 (Project)	24,642
	6311-003 (Project)	67,196
	6076-004 (Project)	104,116
	6077-005 (Project)	56,815
	6309-006 (Project)	11,875
	6079-007 (Project)	59,481
	6078-008 (Project)	33,149
	6310-009 (Project)	24,407
	6307-010 (Project)	16,132
TOTALS		**716,256**
R&R Budget - Other Personnel Funds Requested	6072-001 (Admin Core)	30,925
	6073-001 (Core)	157,662
	6075-002 (Core)	111,214
	6074-003 (Core)	652,722
	6301-001 (Project)	87,115

	6303-002 (Project)	76,452
	6311-003 (Project)	58,980
	6076-004 (Project)	87,957
	6077-005 (Project)	213,060
	6309-006 (Project)	88,481
	6079-007 (Project)	46,386
	6078-008 (Project)	140,149
	6310-009 (Project)	170,033
	6307-010 (Project)	63,167
TOTALS		**1,984,303**
R&R Budget - Section A & B. Total Salary, Wages and Fringe Benefits (A+B)	6072-001 (Admin Core)	91,474
	6073-001 (Core)	206,475
	6075-002 (Core)	116,152
	6074-003 (Core)	730,904
	6301-001 (Project)	213,076
	6303-002 (Project)	101,094
	6311-003 (Project)	126,176
	6076-004 (Project)	192,073
	6077-005 (Project)	269,875
	6309-006 (Project)	100,356
	6079-007 (Project)	105,867
	6078-008 (Project)	173,298
	6310-009 (Project)	194,440

	6307-010 (Project)	79,299
TOTALS		**2,700,559**
R&R Budget - Section C. Total Equipment	6072-001 (Admin Core)	0
	6073-001 (Core)	0
	6075-002 (Core)	0
	6074-003 (Core)	0
	6301-001 (Project)	0
	6303-002 (Project)	0
	6311-003 (Project)	0
	6076-004 (Project)	0
	6077-005 (Project)	0
	6309-006 (Project)	0
	6079-007 (Project)	0
	6078-008 (Project)	0
	6310-009 (Project)	0
	6307-010 (Project)	0
TOTALS		**0**
R&R Budget - Domestic Travel	6072-001 (Admin Core)	18,788
	6073-001 (Core)	5,150
	6075-002 (Core)	0
	6074-003 (Core)	5,150
	6301-001 (Project)	7,500
	6303-002 (Project)	4,000

	6311-003 (Project)	1,500
	6076-004 (Project)	6,000
	6077-005 (Project)	2,000
	6309-006 (Project)	2,499
	6079-007 (Project)	1,500
	6078-008 (Project)	1,500
	6310-009 (Project)	5,238
	6307-010 (Project)	3,000
TOTALS		**63,825**
R&R Budget - Foreign Travel	6072-001 (Admin Core)	0
	6073-001 (Core)	0
	6075-002 (Core)	0
	6074-003 (Core)	0
	6301-001 (Project)	0
	6303-002 (Project)	0
	6311-003 (Project)	0
	6076-004 (Project)	0
	6077-005 (Project)	0
	6309-006 (Project)	0
	6079-007 (Project)	0
	6078-008 (Project)	0
	6310-009 (Project)	0
	6307-010 (Project)	0

TOTALS		**0**
R&R Budget - Section D. Total Travel	6072-001 (Admin Core)	18,788
	6073-001 (Core)	5,150
	6075-002 (Core)	0
	6074-003 (Core)	5,150
	6301-001 (Project)	7,500
	6303-002 (Project)	4,000
	6311-003 (Project)	1,500
	6076-004 (Project)	6,000
	6077-005 (Project)	2,000
	6309-006 (Project)	2,499
	6079-007 (Project)	1,500
	6078-008 (Project)	1,500
	6310-009 (Project)	5,238
	6307-010 (Project)	3,000
TOTALS		**63,825**
R&R Budget - Tuition/Fees/Health Insurance	6072-001 (Admin Core)	0
	6073-001 (Core)	0
	6075-002 (Core)	0
	6074-003 (Core)	0
	6301-001 (Project)	3,450
	6303-002 (Project)	3,000
	6311-003 (Project)	0

	6076-004 (Project)	0
	6077-005 (Project)	0
	6309-006 (Project)	0
	6079-007 (Project)	0
	6078-008 (Project)	0
	6310-009 (Project)	0
	6307-010 (Project)	0
TOTALS		**6,450**
R&R Budget - Stipends	6072-001 (Admin Core)	0
	6073-001 (Core)	0
	6075-002 (Core)	0
	6074-003 (Core)	0
	6301-001 (Project)	0
	6303-002 (Project)	0
	6311-003 (Project)	0
	6076-004 (Project)	0
	6077-005 (Project)	0
	6309-006 (Project)	0
	6079-007 (Project)	0
	6078-008 (Project)	0
	6310-009 (Project)	0
	6307-010 (Project)	0
TOTALS		**0**

R&R Budget - Trainee Travel	6072-001 (Admin Core)	0
	6073-001 (Core)	0
	6075-002 (Core)	0
	6074-003 (Core)	0
	6301-001 (Project)	0
	6303-002 (Project)	0
	6311-003 (Project)	0
	6076-004 (Project)	0
	6077-005 (Project)	0
	6309-006 (Project)	0
	6079-007 (Project)	0
	6078-008 (Project)	0
	6310-009 (Project)	0
	6307-010 (Project)	0
TOTALS		**0**
R&R Budget - Subsistence	6072-001 (Admin Core)	0
	6073-001 (Core)	0
	6075-002 (Core)	0
	6074-003 (Core)	0
	6301-001 (Project)	0
	6303-002 (Project)	0
	6311-003 (Project)	0
	6076-004 (Project)	0

	6077-005 (Project)	0
	6309-006 (Project)	0
	6079-007 (Project)	0
	6078-008 (Project)	0
	6310-009 (Project)	0
	6307-010 (Project)	0
TOTALS		**0**
R&R Budget - Other Participants/Trainee Support Costs	6072-001 (Admin Core)	0
	6073-001 (Core)	0
	6075-002 (Core)	0
	6074-003 (Core)	0
	6301-001 (Project)	0
	6303-002 (Project)	0
	6311-003 (Project)	0
	6076-004 (Project)	0
	6077-005 (Project)	0
	6309-006 (Project)	0
	6079-007 (Project)	0
	6078-008 (Project)	0
	6310-009 (Project)	0
	6307-010 (Project)	0
TOTALS		**0**
R&R Budget - Section E. Total Participants/Trainee Support Costs	6072-001 (Admin Core)	0

	6073-001 (Core)	0
	6075-002 (Core)	0
	6074-003 (Core)	0
	6301-001 (Project)	3,450
	6303-002 (Project)	3,000
	6311-003 (Project)	0
	6076-004 (Project)	0
	6077-005 (Project)	0
	6309-006 (Project)	0
	6079-007 (Project)	0
	6078-008 (Project)	0
	6310-009 (Project)	0
	6307-010 (Project)	0
TOTALS		**6,450**
R&R Budget - Materials and Supplies	6072-001 (Admin Core)	750
	6073-001 (Core)	163,665
	6075-002 (Core)	39,330
	6074-003 (Core)	176,948
	6301-001 (Project)	92,326
	6303-002 (Project)	47,477
	6311-003 (Project)	31,082
	6076-004 (Project)	53,538
	6077-005 (Project)	55,324

	6309-006 (Project)	64,141
	6079-007 (Project)	23,909
	6078-008 (Project)	25,345
	6310-009 (Project)	111,283
	6307-010 (Project)	49,260
TOTALS		**934,378**
R&R Budget - Publication Costs	6072-001 (Admin Core)	1,000
	6073-001 (Core)	0
	6075-002 (Core)	0
	6074-003 (Core)	0
	6301-001 (Project)	2,000
	6303-002 (Project)	2,000
	6311-003 (Project)	1,500
	6076-004 (Project)	3,500
	6077-005 (Project)	2,000
	6309-006 (Project)	1,666
	6079-007 (Project)	1,500
	6078-008 (Project)	3,000
	6310-009 (Project)	0
	6307-010 (Project)	2,000
TOTALS		**20,166**
R&R Budget - Consultant Services	6072-001 (Admin Core)	18,500
	6073-001 (Core)	0

	6075-002 (Core)	0
	6074-003 (Core)	0
	6301-001 (Project)	0
	6303-002 (Project)	0
	6311-003 (Project)	0
	6076-004 (Project)	0
	6077-005 (Project)	0
	6309-006 (Project)	0
	6079-007 (Project)	0
	6078-008 (Project)	0
	6310-009 (Project)	0
	6307-010 (Project)	0
TOTALS		**18,500**
R&R Budget - ADP/Computer Services	6072-001 (Admin Core)	0
	6073-001 (Core)	0
	6075-002 (Core)	0
	6074-003 (Core)	0
	6301-001 (Project)	0
	6303-002 (Project)	0
	6311-003 (Project)	0
	6076-004 (Project)	0
	6077-005 (Project)	0
	6309-006 (Project)	0

	6079-007 (Project)	0
	6078-008 (Project)	0
	6310-009 (Project)	0
	6307-010 (Project)	0
TOTALS		**0**
R&R Budget - Subawards/Consortium/Contractual Costs	6072-001 (Admin Core)	0
	6073-001 (Core)	0
	6075-002 (Core)	0
	6074-003 (Core)	0
	6301-001 (Project)	0
	6303-002 (Project)	0
	6311-003 (Project)	0
	6076-004 (Project)	0
	6077-005 (Project)	0
	6309-006 (Project)	0
	6079-007 (Project)	0
	6078-008 (Project)	0
	6310-009 (Project)	0
	6307-010 (Project)	0
TOTALS		**0**
R&R Budget - Equipment or Facility Rental User Fees	6072-001 (Admin Core)	0
	6073-001 (Core)	0
	6075-002 (Core)	0

	6074-003 (Core)	0
	6301-001 (Project)	0
	6303-002 (Project)	0
	6311-003 (Project)	0
	6076-004 (Project)	0
	6077-005 (Project)	0
	6309-006 (Project)	0
	6079-007 (Project)	0
	6078-008 (Project)	0
	6310-009 (Project)	0
	6307-010 (Project)	0
TOTALS		**0**
R&R Budget - Alterations and Renovations	6072-001 (Admin Core)	0
	6073-001 (Core)	0
	6075-002 (Core)	0
	6074-003 (Core)	0
	6301-001 (Project)	0
	6303-002 (Project)	0
	6311-003 (Project)	0
	6076-004 (Project)	0
	6077-005 (Project)	0
	6309-006 (Project)	0
	6079-007 (Project)	0

	6078-008 (Project)	0
	6310-009 (Project)	0
	6307-010 (Project)	0
TOTALS		**0**
R&R Budget - Other Direct Cost 1	6072-001 (Admin Core)	1,480
	6073-001 (Core)	23,347
	6075-002 (Core)	5,823
	6074-003 (Core)	40,029
	6301-001 (Project)	34,990
	6303-002 (Project)	5,000
	6311-003 (Project)	0
	6076-004 (Project)	600
	6077-005 (Project)	4,000
	6309-006 (Project)	4,998
	6079-007 (Project)	1,250
	6078-008 (Project)	0
	6310-009 (Project)	6,511
	6307-010 (Project)	26,500
TOTALS		**154,528**
R&R Budget - Other Direct Cost 2	6072-001 (Admin Core)	9,600
	6073-001 (Core)	78,486
	6075-002 (Core)	695
	6074-003 (Core)	0

	6301-001 (Project)	5,621
	6303-002 (Project)	4,500
	6311-003 (Project)	0
	6076-004 (Project)	24,150
	6077-005 (Project)	0
	6309-006 (Project)	4,165
	6079-007 (Project)	5,250
	6078-008 (Project)	0
	6310-009 (Project)	0
	6307-010 (Project)	0
TOTALS		**132,467**
R&R Budget - Other Direct Cost 3	6072-001 (Admin Core)	150
	6073-001 (Core)	0
	6075-002 (Core)	18,000
	6074-003 (Core)	0
	6301-001 (Project)	6,000
	6303-002 (Project)	0
	6311-003 (Project)	0
	6076-004 (Project)	10,500
	6077-005 (Project)	0
	6309-006 (Project)	53,895
	6079-007 (Project)	0
	6078-008 (Project)	0

	6310-009 (Project)	0
	6307-010 (Project)	0
TOTALS		**88,545**
R&R Budget - Section F. Total Other Direct Cost	6072-001 (Admin Core)	31,480
	6073-001 (Core)	265,498
	6075-002 (Core)	63,848
	6074-003 (Core)	216,977
	6301-001 (Project)	140,937
	6303-002 (Project)	58,977
	6311-003 (Project)	32,582
	6076-004 (Project)	92,288
	6077-005 (Project)	61,324
	6309-006 (Project)	128,865
	6079-007 (Project)	31,909
	6078-008 (Project)	28,345
	6310-009 (Project)	117,794
	6307-010 (Project)	77,760
TOTALS		**1,348,584**
R&R Budget - Section G. Total Direct Cost (A thru F)	6072-001 (Admin Core)	141,742
	6073-001 (Core)	477,123
	6075-002 (Core)	180,000
	6074-003 (Core)	953,031
	6301-001 (Project)	364,963

	6303-002 (Project)	167,071
	6311-003 (Project)	160,258
	6076-004 (Project)	290,361
	6077-005 (Project)	333,199
	6309-006 (Project)	231,720
	6079-007 (Project)	139,276
	6078-008 (Project)	203,143
	6310-009 (Project)	317,472
	6307-010 (Project)	160,059
TOTALS		**4,119,418**
R&R Budget - Section H. Indirect Costs	6072-001 (Admin Core)	68,745
	6073-001 (Core)	405,450
	6075-002 (Core)	141,300
	6074-003 (Core)	1,287,330
	6301-001 (Project)	200,640
	6303-002 (Project)	91,059
	6311-003 (Project)	86,539
	6076-004 (Project)	168,409
	6077-005 (Project)	179,928
	6309-006 (Project)	121,653
	6079-007 (Project)	67,549
	6078-008 (Project)	117,823
	6310-009 (Project)	355,317

	6307-010 (Project)	88,833
TOTALS		**3,380,575**
R&R Budget - Section I. Total Direct and Indirect Costs (G +H)	6072-001 (Admin Core)	210,487
	6073-001 (Core)	882,573
	6075-002 (Core)	321,300
	6074-003 (Core)	2,240,361
	6301-001 (Project)	565,603
	6303-002 (Project)	258,130
	6311-003 (Project)	246,797
	6076-004 (Project)	458,770
	6077-005 (Project)	513,127
	6309-006 (Project)	353,373
	6079-007 (Project)	206,825
	6078-008 (Project)	320,966
	6310-009 (Project)	672,789
	6307-010 (Project)	248,892
TOTALS		**7,499,993**

A. COMPONENT COVER PAGE

Project Title: Administrative Core
Component Project Lead Information: WHITLEY, RICHARD J.

B. COMPONENT ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

SPECIFIC AIMS:
The Administrative Core of the Antiviral Drug Discovery and Development Center (AD3C) has provided organizational leadership for the current CETR and will continue to play a key role in leadership, communication, coordination and oversight of the proposed projects and cores. Importantly, it will promote collaboration and synergy between the projects. Operationally, it is in charge of fiscal and contractual management of the center and organizes activities such as meetings of the Executive Committee (EC), Scientific Advisory Committee (SAC), and an annual meeting of all projects and cores. In addition, it will manage the inter-institutional consortium/contractual agreements. The broad objectives of the core are as follows:

1. Providing programmatic and administrative leadership:
Make decisions, specifically “go versus no-go” decisions per discussions with the EC; Track and encourage research productivity; Promote interactions and collaboration between projects and cores, in particular to facilitate overarching synergy to pursue broad-spectrum antivirals; Monitor the direction and overall priorities of the Center; and Directly interface with NIH staff and consultants

2. Fiscal and administrative management of the center:
Finances: oversee expenditures, budget information, fiscal reports; Manage contracts and the consortium agreement; Establish and monitor compliance with federal and NIH regulations

3. Develop, support and monitor progress of projects:
Manage projects through regular conference calls and in-person meetings; Organize semi-annual project reviews (face to face or teleconference) by the EC; Monitor overall Center research quality and progress annually by the SAC; Solicit additional regulatory guidance on an as-needed basis; and Assist with identification and management of intellectual property developed by projects

4. Stimulate collaboration and synergy
Identify potential areas or topics of collaborations between projects and cores; Provide and facilitate access to resources needed in the projects; Ensure that all active compounds are cross-evaluated in other projects; Manage a data sharing and project tracking web-based system; Encourage public/private partnerships and alternative sources of funding to expedite project development and translation to INDs

5. Facilitate meetings
Host monthly conference calls of project teams; Organize conference call-based and in-person meetings of the project and core PIs (EC); Organize and implement an annual face to face meeting with all involved personnel, including the SAC; Facilitate consulting and other scientific and professional meetings; Attend the NIH reverse site visit

6. Outreach to the public:
Maintain a website; Manage press releases; and Assist with scientific publications and ensure grant acknowledgement

We firmly believe that a strong Administrative Core will be crucial to the success of the AD3C. We have brought together leading virologists, chemists, scientific advisors and projects and cores from around the country and are confident that the Administrative Core will facilitate communication and collaboration between the PIs. Accomplishing the objectives set out above will ensure focus and synergy among the projects, accelerating the development of new, potentially broad-spectrum therapeutics for (re-)emerging infections of coronaviruses, alphaviruses, flaviviruses and influenza.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: B.2 Admin Core Report.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

Not Applicable

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

NOTHING TO REPORT

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

This next year, the major goal of the administrative core is to continue to be a successful mediator for the interaction and synergy among all groups. We will continue to hold all monthly meetings and strive to ensure each project and core has what they need to be productive

in meeting milestones. Keeping all project and core leaders informed and focused is the major goal of the administrative core.

We received feedback from the scientific advisory committee on some clear goals to improve such as producing via the science cores a master spreadsheet profiling the compounds relative data across all projects. We will work to implement the suggested changes.

Administrative Core
RPPR materials Y1

B.2 *What was accomplished under these goals?*

The administrative core is in place to serve as a primary coordinator between all sites. The objectives are listed below with the year 1 accomplishments highlighted under each goal:

1. **Providing programmatic and administrative leadership**
   - One major accomplishment is that addendum to the consortium agreement from the previous award was signed by all sites and provides a means of governing shared data and materials.
   - Over the year 1 period, the administrative core has been responsible for scheduling monthly teleconference calls to facilitate constant updates among groups. This core ensures that all groups present updates and/or issues that arise so that group leaders can stay in touch with each other and aid in problem solving. Meeting minutes are recorded, sent to groups, and action items are checked on post meetings to assess progress or identify issues.
2. **Fiscal and administrative management of the center**
   - All budgets and agreements have been managed under the administrative core.
3. **Develop, support and monitor progress of projects**
   - Timelines were produced for each project and milestones are discussed during monthly calls. The administrative core prompted all groups to develop the timelines and keep them updated.
4. **Stimulate collaboration and synergy**
   - Teleconference meetings are well attended by all group leaders.
   - The scientific cores at Southern Research were great hubs to facilitate collaboration and synergy. These positive interactions are mediated via the administrative cores or by the project and group leaders.
5. **Facilitate meetings**
   - Monthly calls were hosted via teleconference service (AT&T)
   - Administrative core hosted a very successful AD3C in person meeting in Birmingham, AL for each project and core to present the status of each project to the scientific advisory committee. This meeting was very well received by everyone and the structure was modified so that data produced via core leaders was combined into the project presentations. This format change from previous years was welcomed by groups and required more collaboration and synergy from cores with projects that will serve the purpose of the meeting in years to come. The meeting was well attended by all core and project groups. The success of the meeting is echoed in the scientific advisory committee report.
6. **Outreach to the public**

(b) (6), (b) (3) (A)

C. COMPONENT PRODUCTS

C.1 PUBLICATIONS

Not Applicable

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Not Applicable

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Not Applicable

C.5 OTHER PRODUCTS AND RESOURCE SHARING

Nothing to report

D. COMPONENT PARTICIPANTS

Not Applicable

E. COMPONENT IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

Not Applicable

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

NOTHING TO REPORT

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?
Not Applicable

F. COMPONENT CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

During the Y1 reporting period, the administrative core experienced turnover of personnel that resulted in some challenges. The change was handled well due to the continuous attention to organization that the administrative core had and continues to maintain. We do not anticipate personnel changes to result in major problems for Y2.

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. COMPONENT SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

Not Applicable

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Not Applicable

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT

Not Applicable

G.10 ESTIMATED UNOBLIGATED BALANCE

Not Applicable

G.11 PROGRAM INCOME

Not Applicable

G.12 F&A COSTS

Not Applicable

RESEARCH & RELATED BUDGET - SECTION A & B FINAL

ORGANIZATIONAL DUNS*: 063690705

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2020 **End Date*:** 02-28-2021

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr	Richard	J	Whitley		Project Director/ Principal Investigator	0.00	(b) (6)			19,230.00	5,423.00	24,653.00
2.		(b) (6), (b) (3) (A)				Research Project Director	0.00				28,000.00	7,896.00	35,896.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **60,549.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
1	Secretarial/Clerical	(b) (6)			6,814.00	2,235.00	9,049.00
1	Program Manager				16,473.00	5,403.00	21,876.00
2	**Total Number Other Personnel**					**Total Other Personnel**	**30,925.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**91,474.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E

ORGANIZATIONAL DUNS*: 063690705

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2020 **End Date*:** 02-28-2021

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	18,788.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**18,788.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		0.00
2. Stipends		0.00
3. Travel		0.00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K

ORGANIZATIONAL DUNS*: 063690705

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2020 **End Date*:** 02-28-2021

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	750.00
2. Publication Costs	1,000.00
3. Consultant Services	18,500.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8. Website and Monthly Teleconference Calls	1,480.00
9. Annual Meeting Expenses	9,600.00
10. Postage and Shipping	150.00
Total Other Direct Costs	**31,480.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**141,742.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. MDTC	48.5	141,742.00	68,745.00
		Total Indirect Costs	**68,745.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number)	Department of Health and Human Services (DHHS) Steven Zuraf (301) 492-4855

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**210,487.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification*	File Name: Admin Core Budget Justification Yr 2 12-12-2019.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

WHITLEY -UAB ADMIN CORE

BUDGET JUSTIFICATION

Personnel

Richard J. Whitley, MD – Core Lead (b) (6) calendar months (b) (6), Year 2 in addition to effort on Project 4) Dr. Whitley is currently Professor of Pediatrics, Microbiology, Medicine and Neurosurgery, and holds the Loeb Chair in Pediatrics in the School of Medicine at the University of Alabama at Birmingham (UAB). He has performed translational research for more than 40 years at UAB and has focused on antiviral drug development ranging from molecule selection, preclinical toxicology, ADME, animal models, resistance assessment, to human studies. As the PD/PI of AD3C, Dr. Whitley will continue to serve as the Administrative Core Lead, and will utilize his expertise in managing consortia with multiple institutions to ensure appropriate communications between projects and cores and will aid in conflict resolution, should any conflicts arise.

(b) (6), (b) (3) (A) **PhD - Administrative Core Associate Director (b) (6) calendar months effort – Year 2)**

(b) (6), (b) (3) (A) recently joined the Administrative Core as associate director. Prior to beginning work with the AD3C, (b) (6), (b) (3) (A) directed a research lab in the (b) (6), (b) (3) (A) for almost (b) (6), (b) (3) (A) years, and also has a broad range of experience through pervious work on projects in Biochemistry, Biophysics and Pathology. (b) (6), (b) (3) (A) will coordinate the activities of the necessary project teams ensuring the appropriate expertise is represented and will organize and chair the various project team meetings. In addition, (b) (6), (b) (3) (A) will work with AD3C's PI and Core Lead (Richard Whitley – see above) and Business Officer (b) (6), (b) (3) (A) – see below) to achieve the Administrative Core's objectives. These include the organization of all in-person meetings, communication with the Executive Committee and Scientific Advisory Committee, communication with NIAID, preparation of relevant documents such as annual progress reports, solicitation and organization of scientific review of supplemental research projects, and maintaining AD3C's website.

(b) (6), (b) (3) (A) **– Administrative Core Business Officer ((b) (6) calendar months effort -Year 2)**

(b) (6), (b) (3) (A) has more than (b) (6), (b) (3) (A) years in research administration of federal grants and contracts, and has been administrative manager for the multisite clinical trials group (CASG) for more than (b) (6), (b) (3) (A) years. (b) (6), (b) (3) (A) is the current AD3C program manager, and will continue to oversee the financial and contractual administration of the AD3C. (b) (6), (b) (3) (A) will be responsible for all budgetary issues including institutional consortium/cooperative agreements, subawards, amendments to the budget, invoicing, monitoring spending, and keeping the PI and Executive Committee informed about the status of the annual budget. (b) (6), (b) (3) (A) will be responsible for grants management issues and will aid (b) (6), (b) (3) (A) (see above) with meeting organization, the progress report preparation, and all other proposals related to AD3C funding. (b) (6), (b) (3) (A) will oversee all compliance and federal regulatory requirements of AD3C.

Other Administrative Staff

Other Administrative Staff includes (b) (6), (b) (3) (A) ((b) (6) **calendar months – Year 2**) who is the administrative coordinator for Dr. Rich Whitley. (b) (6), (b) (3) (A) will continue to assist Drs. Whitley and (b) (6), (b) (3) with logistical aspects of meeting scheduling, travel arrangements, and organization as well as maintenance of the website.

Consultants -

Total Funds **($18,500 Year 2**) are budgeted for five members of the required **Scientific Advisory Committee (SAC)** who attend the annual AD3C meeting and review activities of all projects. One member will also attend the NIH reverse site visit. As projects continue to progress through the product development process, their continued guidance, oversight, and recommendations for the four projects will be of critical importance. The budget is based on estimated $3,500 reimbursement per each of the 5 advisors annually including travel costs and consultant fee/honorarium. In addition**, $1,000** is budgeted to cover costs of a **Consultant** to provide additional expert advice and guidance on regulatory issues for Center projects.

Supplies

Minimal funding of **$750 (Year 2)** is requested to provide for copier, postage, and office supplies needed to manage administrative activities of the large multi project program including supplies for the annual meeting. Costs are estimated based on historical experience with similar multi-site project management.

Travel

Funds **($19,200- Year 2**) are requested for the following travel:

- Annual NIH reverse site visit expenses for 5 people (the PD/PI, Administrative Director, 2 project leaders, and SAC member the Rockville, MD area. **($5,000)**
- PD/PI, core personnel and/or Administrative Director travel to provide technical assistance or oversight to one or more projects **($1500**)
- Executive committee meeting for Core and Project leaders held approximately six months after Center Annual meeting ($**6700**).
- Travel ($**4,000**) for the Core Leader and Administrative Associate to attend annual national or international research conferences such as International Conference on Antiviral Research (ICAR) or Drug Development symposia to present research and collaborate with other investigators in the area.

Other Direct Expenses - $12,230 (Year 2)

Teleconference/web meeting- $750 Funds will be used to cover the costs of monthly teleconferences and/or web meetings outlined in the Administrative Core plan to review and monitor projects and update project leaders on a continuous basis.

Computer website maintenance and update ($730): costs include website revisions and updates to an existing UAB site developed for drug discovery and development research. The website allows for communication and data sharing among investigators as well as providing a means for public access.

Publication/duplication costs ($1,000): Funding is requested to cover costs related to publications of manuscripts and copying of draft progress and final reports, meeting minutes, and material used for annual or executive committee meetings. Costs are based on historical costs for meeting and reports used for a similar program.

Postage/shipping ($150): funding for mailing or shipping of Center related items to sites, NIH and/or external institutions.

Annual meeting expenses ($9,600): funds are included to cover costs related to hosting the large Center annual meeting for investigators and scientific advisors for two days. The budget includes costs for meeting space, audiovisual services, incidental refreshments and meals for attendees, and rental of items such as poster display boards.

Indirect Costs

Indirect Costs are assessed at the approved UAB federal F&A rate of 48.5% on a modified total direct costs basis.

A. COMPONENT COVER PAGE

Project Title: Assay Core

Component Project Lead Information:

Bostwick, James Robert

B. COMPONENT ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

Working in conjunction with investigators from all four projects, the Assay Core at Southern Research, directed by Dr. Robert Bostwick, will accomplish the following work:

Specific Aims

Aim 1: Measure potency and efficacy of compounds synthesized by the MCLDC to determine SAR and drive compound optimization. The AC will test all compounds synthesized in this program using in vitro assays already developed and established in the previous U19 grant. Three assays will be employed for each virus as follows: an antiviral (AV) assay to measure compound potency (EC50), a virus titer reduction (VTR) assay to measure compound efficacy (maximum log fold-reduction), and a cell viability assay to measure compound cytotoxicity (CC50). The combination of these three values will be used by the MCLDC to drive SAR for further compound optimization in iterative design-make-test cycles and to select compounds for additional testing in secondary assays conducted by the project teams.

Aim 2: Identify additional compounds with antiviral activity for each virus by testing a collection composed of 800 nucleosides and nucleotides. In order to identify chemical assets with potential for development as prodrug compounds, each compound in this collection will be tested in the three assays listed in Aim 1 for each virus. Compounds with antiviral effects will be selected for further testing in secondary assays by the project teams.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: 191212_AssayCoreB2_SM.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

Not Applicable

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

NOTHING TO REPORT

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

Aim 1: All compounds synthesized by the MCLDC for all projects will be screened in relevant assays as required.
Aim 2: Compound collections will be screened to identify new chemical leads for each project as required.

Core B: Assay Core
RPPR U19 AD3C Y1
B2: Accomplishments under these goals

Aim 1 is ongoing work for the duration of the grant. The MCLDC needs data measuring both potency and efficacy of compounds in order to develop SAR for each chemical series. Accordingly, the Assay Core utilizes plate-based assays executed on automated robotic platforms to enable testing of all the compounds that are synthesized. Each assay has been customized for each virus and utilizes different assay readouts as appropriate. Two SAR driving assays (in addition to a cytotoxicity assay) are used for each virus target to support chemistry efforts. One assay reports the % inhibition of virus effect in the host cell and is used to measure compound potency. The other assay measures the fold log reduction of virus titer in selected wells from the potency assay and is used to measure compound efficacy. The virus titer reduction data is obtained using media from the assay plates for which virus potency is determined so that the anti-viral effect of a compound is measured both in terms of ability to inhibit virus effects in the host cell as well as reduction in virus titer in the same assay well. Table 1 summarizes the number of compounds tested in each assay for each project during the first nine months of year one (March through November, 2019).

Table 1. Summary of Assays used for SAR

Project #	Virus	Cell line	Anti-viral (AV) Potency Assay	# Compounds tested*	Virus Titer Reduction (VTR) Efficacy Assay	# Compounds tested*
1	SARS	Vero E6	VLNLR	26	VLNLR	
2	DENV	HEK293	IFA	69	CPE	
2	ZIKV	Vero CCL81	IFA	120	CPE	
3	CHIKV	THF	VLNLR	221	VLNLR	32
3	VEEV	THF	CPE	221	CPE	6
4	H3N2/H1N1	HEK293	IFA	154	ELVIRA	

* March-November, 2019

Assay Abbreviations

IFA Immunofluorescence assay (Mirrorball)
VLNLR Virus linked nanoluc luciferase reporter assay
CPE Cytopathic Effect assay
ELVIRA Enzyme linked virus inhibitor reporter assay

Aim 2 specified work has not been initiated because the Assay Core at SR has not received the compound collection. Instead, two HTS campaigns have been conducted using a different compound library than was previously screened in order to identify additional hits needed for projects 3 (DENV) and 4 (Influenza) (See section F). Details for each project are described in Core Specific information below.

C. COMPONENT PRODUCTS

C.1 PUBLICATIONS

Not Applicable

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Not Applicable

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Not Applicable

C.5 OTHER PRODUCTS AND RESOURCE SHARING

Nothing to report

D. COMPONENT PARTICIPANTS

Not Applicable

E. COMPONENT IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

Not Applicable

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

NOTHING TO REPORT

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?
Not Applicable

F. COMPONENT CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. COMPONENT SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

Not Applicable

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Not Applicable

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT

Not Applicable

G.10 ESTIMATED UNOBLIGATED BALANCE

Not Applicable

G.11 PROGRAM INCOME

Not Applicable

G.12 F&A COSTS

Not Applicable

RPPR - Core-6073

RESEARCH & RELATED BUDGET - SECTION A & B **FINAL**

ORGANIZATIONAL DUNS*: 0069005260000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: Southern Research Institute

Start Date*: 03-01-2020 **End Date*:** 02-28-2021

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	James	Robert	Bostwick		Core Lead	0.00	(b) (6)			34,207.00	14,606.00	48,813.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **48,813.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
6	3 Scientist/2 Data Analyst/1Supvr Comp Mgnt	(b) (6)			110,485.00	47,177.00	157,662.00
6	**Total Number Other Personnel**					**Total Other Personnel**	**157,662.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**206,475.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E

ORGANIZATIONAL DUNS*: 0069005260000
Budget Type*: ● Project ❍ Subaward/Consortium
Enter name of Organization: Southern Research Institute

Start Date*: 03-01-2020 **End Date*:** 02-28-2021

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	5,150.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**5,150.00**

E. Participant/Trainee Support Costs		Funds Requested ($)*
1. Tuition/Fees/Health Insurance		0.00
2. Stipends		0.00
3. Travel		0.00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K

ORGANIZATIONAL DUNS*: 0069005260000
Budget Type*: ● Project ○ Subaward/Consortium
Enter name of Organization: Southern Research Institute

Start Date*: 03-01-2020 **End Date*:** 02-28-2021

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	163,665.00
2. Publication Costs	0.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8. BSL3 charges	23,347.00
9. Other charges	78,486.00
Total Other Direct Costs	**265,498.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**477,123.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. OH-Salaries + Benefits	120.0	206,475.00	247,770.00
2. G&A - Salaries + Benefits + OH + ODC	20.0	724,893.00	144,979.00
3. FCCM OH	5.8	206,475.00	11,976.00
4. FCCM G & A (Actual rate is 0.10%)	1.0	724,893.00	725.00
		Total Indirect Costs	**405,450.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number) DHHS / Darryl Mayes ? 301-492-4852

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**882,573.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification* File Name:
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

A. COMPONENT COVER PAGE

Project Title: Core C.1 Medicinal Chemistry and Lead Development Core - SR
Component Project Lead Information: PATHAK, ASHISH KUMAR

B. COMPONENT ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

Primary responsibilities for Core C (MCLDC) of the AD3C include: 1) Hit-to-lead and lead optimization chemistry through structure-activity relationship (SAR) analysis on reconfirmed high-throughput screening (HTS) hits; 2) absorption, distribution, metabolism, and excretion (ADME) and pharmacokinetic (PK) analysis; 3) structural biology; 4) structure-based virtual screening of commercial libraries against relevant X-ray-derived models; 5) analysis of additional HTS data against influenza and Dengue viruses; and 6) purchase or resynthesize promising hits as needed for potential follow-up chemistry, and distribute to the appropriate Research Projects for evaluation in secondary assays. In continuation with the chemistry activities performed in Year 5 of the previous grant, the MCLDC continued doing all of these activities. The Compound Management Group continued to store (-20oC) and distribute all samples to various Research Project sites for additional antiviral analysis using the proper handling and shipping methods for compounds. Compound Management has also been responsible for maintaining the (b) (3) (A), Database in which unique compound identification numbers (SRI Number) are provided and all biological and ADME data for each compound is captured for easy retrieval. The Bioanalytical Group generated all in vitro ADME data which is also captured in (b) (3) (A), system and easily accessible for retrieval and analysis by medicinal chemists. Medicinal chemistry on active HTS hits Dengue virus (DENV), ZIKA Virus (ZIKAV), Severe Acute Respiratory Syndrome (SARS) virus, Chikungunya virus (CHIKV), Venezuelan equine encephalitis virus (VEEV) and Influenza virus] was continued in Yr. 1 of the current AD3C program. Several closely-related analogs were also acquired from commercial sources for reconfirmation of activity and initial SAR activities. All compounds were analyzed for purity by HPLC and integrity by 1H NMR and HR-MS before registering in (b) (3) (A). Synthesis of analogs for each project was prioritized based on the stage of the hit-to-lead optimization and the availability of antiviral assays.
The Specific Aims of Core C, which remain unchanged, are:
Aim 1: Provide lead generation and optimization through medicinal chemistry efforts, SAR studies, structural biology and computational chemistry analyses, and ADME and pharmacokinetic (PK) data to support compound progression into in vivo antiviral efficacy models at Research Project sites. Iterative design-synthesis and testing in SAR assays will be carried out to optimize compounds identified from the current CETR grant. Parameters to optimize will be antiviral potency and efficacy (EC90 < 1 μM; VTR > 3 logs @ 10 μM), cytotoxicity (CC50 > 30 μM) and drug-like properties, such as mouse liver microsomal stability (MLM t½ >60 min) and solubility (>10 μM). Structural biology and computational approaches in drug design will also be utilized, in particular for projects that already have target(s) identified. Nucleoside-based RdRp inhibitors will also be developed along with compounds from previous HTS screens for all viruses. Antiviral activity from a unique collection of 800 nucleosides and nucleoside prodrugs from our partner, EIDD, will also be analyzed. Any hits identified from the Assay Core will go through the iterative design-synthesis process and any compound which meets the antiviral potency and efficacy criteria will be evaluated for in vivo bioavailability and selected for animal studies. Compounds with adequate plasma levels will be tested in vivo. The MCLDC will be responsible for all phases of compound optimization and subsequent synthetic scale-up leading to the in vivo potency studies.
Aim 2: Provide integrated informatics support which includes compound tracking, as well as data capture, analysis, storage, backup and retrieval. Informatics support is an integral part of any drug discovery process, especially for a drug discovery program of this size since a large number of compounds will be synthesized and tested in variety of assays. Careful tracking of compounds submitted to the Assay Core for testing in the primary SAR screen as well as coordination of data generated from the Research Project sites will be important. Compounds coming from Emory Institute for Drug Development (EIDD) will also be distributed to the corresponding Research Project sites via SR's compound management group. SR will use a secured, access-limited, password-protected online file-sharing system (b) (3) (A), controlled by Southern Research for data sharing and storage for immediate access to collaborators spanning the various Research Project and Core sites.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: 191212_MedChemCoreSR_B.2_SM.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

Not Applicable

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

File uploaded: B.4 Med Chem Core SR.pdf

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

During Year 2 period of the project, we will continue with our efforts to perform hit-to-lead and lead optimization chemistry approaches on two chemical series (one main and one back-up) in each of the CHIKV and VEEV projects and on one each chemical series for the SARS, ZIKA, DENV and Influenza projects. Our aim is to generate at least three hit to lead compounds with reasonable ADME and in vivo PK properties that can be evaluated for potency and efficacy in a mouse model as a proof of concept study. These leads will be

further optimized with the goal of identifying a pre-clinical candidate(s).

Research Performance Progress Report
Core C: Medicinal Chemistry and Lead Development Core (MCLDC) at Southern Research
Section B.2

The Core C performed in all of the primary responsibilities. Compound Management Group carried out compound repository and all samples were stored in freezers before shipping to various Research Projects locations according to compounds handling and shipping methods. This group is also responsible for maintaining the (b) (3) (A), (b) (4) Compound Management Database provided unique compound identification numbers (SRI Number) as well as used to store biological and ADME data. ADME and Analytical Group carried out *in vitro* ADME analysis and stored data in the (b) (3) (A), (b) (4) system for retrieval and analysis by medicinal chemists. This group also carried out high resolution exact mass (HR-MS) analysis and sample purity by HPLC.

Chemical synthesis work in Core C for different projects were prioritized with limited number of bench chemists based on the stages of compound development in hit-to-lead or lead optimization processes as well as availability of different antiviral assays. Medicinal chemistry approaches consist of several steps such as hit(s) identification from HTS data, hits re-confirmation, preliminary ADME properties and visual inspection of structures for uniqueness to prioritize the processes of hit selection and hit-to-lead after which analogs are then synthesized to generate SAR. A lead is then generated to further pursue lead optimization where activity and PK properties are optimized towards the identification of a candidate for evaluation in an animal efficacy model.

Research Project-1 on Coronaviruses includes Severe Acute Respiratory Syndrome (SARS) virus. Lead identification and optimization on previously re-confirmed HTS hit SRI-36097 was carried out. Thus far 45 unique analogs were synthesized and screened for its potency in the NanoLuc (NL) assay developed by Assay Development Core (Core B) as the primary SAR assay and cytotoxicity assay to develop preliminary SAR information. The *in vitro* ADME properties of active compounds within each series were also determined. Some of these compounds with virus plaque reduction >2 logs in VeroE6 cells were further tested in a virus plaque reduction assay using HAE cultures at Research Project-2 site. Results of these studies are provided in Section G (Core Specific Information). Recently, five additional potent re-confirmed hits from NanoLuc-SAR assay were selected for testing in HAE cultures as well as virus titer reduction assays and submitted to Research Project sites.

Research Project-2 on Alphaviruses includes VEE and CHIK viruses. Core C continued chemistry on lead generation and optimization studies on HTS CHIKV hit SRI-33001 and SRI-36767. The compounds were tested for antiviral potency (EC_{90}) and efficacy (VTR) at HTS using CPE-VTR combo assay followed by reconfirmation assay in Research Project-2 laboratory. ADME properties, such as aqueous solubility, log D, and mouse and human microsomal stability, were evaluated on all active compounds. As a result of antiviral data on analogs of SRI-33001, SRI-41627 emerged as a new lead and was submitted for *in vivo* PK studies followed by *in vivo* toxicity and efficacy studies in C57BL/6 mice. Results of these studies are provided in Section G (Core Specific Information). In parallel to the SRI-33001 series, SAR studies were carried out on the HTS re-confirmed hit, SRI-36767. So far, 96 unique analogs were synthesized and submitted to Core B to screen in a combination assay of antiviral activity (EC_{50}) and VTR. New analogs were submitted to HTS to run in NanoLuc-VTR combo assay and to the reconfirmation assay in Research Project-2 laboratory. ADME properties, such as aqueous solubility, log D, and mouse and human microsomal stability, were evaluated on active compounds. This is one of the most advanced series among all of the research projects as one of the potent analog, SRI-42718, was nominated for *in vivo* efficacy studies based on its PK properties. This compound showed excellent activity *in vivo* and further evaluations are ongoing. Results of these studies are provided in Section G (Core Specific Information).

Lead generation studies on hit, SRI-36426, which was identified as a VEEV and CHIKV dual inhibitor continued in Yr.1 of currently funded AD3C. A total of 80 diverse analogs were designed, synthesized and submitted to Core B to screen in a combination assay of antiviral effect (EC_{50}) and VTR. Active compounds were then sent to the Research Project Site-3 and tested in a VTR assay (@ 10 µM) followed by EC_{90} values. A promising lead, SRI-42776 was identified for VEEV and nominated to *in vivo* PK studies. Results of these studies are provided in Section G (Core Specific Information). Recently, lead generation studies also began on a new CHIKV HTS hits SRI-43921 as to have additional backup compound for *in vivo* efficacy studies. This compound did not show any activity against host targets hDHODH and HO1. Results of these studies are provided in Section G (Core Specific Information).

In addition to small molecule antivirals, six nucleosides hits from various HTS screens run for CHIKV, VEEV, DENV, ZIKA and influenza were also tested for antiviral activity. Three nucleosides, SRI-7988, SRI-18457 and SRI-12101 were active against alphaviruses (CHIKV and VEEV), flaviviruses (DENV, WNV, ZIKAV) and

influenza virus. Nucleoside 6-aza-uridine (6-Aza-U, SRI-7988) reconfirmed antiviral activity against alphaviruses and flaviviruses and showed no cytotoxicity against HEK293 and NHDF cells. A prodrug strategy was exploited on 6-Aza-U (SRI-7988) to increase lipophilicity and tissue uptake, and improve drug-like properties and antiviral activity. In addition, 6-aza-cytosine (6-aza-C, SRI-10813) was also synthesized and tested for antiviral activity against alpha-, flavi- and influenza viruses and was found to be selectively active against alphaviruses. Results of these studies are provided in Section G (Core Specific Information).

Structural biology and computational chemistry. ADP-ribose binding site at the macrodomain of nsP3 has been shown to be critical for CHIKV virus replication and infection. The crystal structures of nsP3 macrodomain with and without ADP-ribose (ADPR) were previously determined beyond 1.6Å here at Southern Research. In Yr. 1, fragments obtained from virtual screening were soaked in the nsP3 macrodomain crystals and structures were determined. So far, there are only three fragments from 1st virtual screening that were identified in the ADP-ribose binding site on the nsP3 macrodomain. Co-crystal structures of all 8 fragments in the nsP3 of CHIKV were generated. These fragments were also docked to crystal structures of VEEV and MERS nsP3 macrodomain which have conserved binding site. Results of these studies are provided in Section G (Core Specific Information).

Research Project-3 on Flaviviruses include Dengue virus (DENV) and Zika virus (ZIKAV). Lead generation studies on two reconfirmed hits against ZIKA virus, SRI-39748 and SRI-39757 that were identified from the CPE-based ZIKAV HTS screen of 310K compounds collection is ongoing. The current focus of the chemistry on SRI-39748 and SRI-39757 is to generate modified novel scaffolds with antiviral activity. The compounds were tested in a Mirror Ball (MB) immunofluorescent (IF) assay (SAR assay) which measures M2 protein levels of virus and cytotoxicity. All compounds were also sent to Research Project-3 site to be tested for antiviral activity (EC_{90}) and efficacy (VTR). ADME properties of active compounds within each chemical series are also determined. Results of these studies are provided in Section G (Core Specific Information). As described in project-2, nucleoside SRI-7988 and its synthesized 5'-prodrugs were also tested against DENV-2 and three prodrugs SRI-43846, SRI-43918 and SRI-44020 showed antiviral activity in a VTR assay. These three compounds were further tested against DENV-1, 2, 3 and 4 strains in a replicon assay and found to be active against all 4 strains. SRI-43846 was nominated for *in vivo* PK studies (IV, PO and IP routes of administration). Results of these studies are provided in Section G (Core Specific Information).

Research Project-4 on Influenza viruses includes three strains (H1N1, H3N2 and H5N1). Assay Core (Core B) completed a new HTS campaign on a library 200K compounds from ChemDiv which were not screened before using a Mirror Ball (MB) immunofluorescent (IF) assay to measure M2 protein levels in H3N2-infected A549 cells. The HTS campaign provided 140 hit compounds which were analyzed for structural liabilities using the Pan Assay Interference Compounds (PAINS) filter and visual inspection from which 31 compounds were selected for reconfirmation in antiviral and cell cytotoxicity assays and commercial samples were acquired. Of these 31 hits, 26 compounds passed the structural integrity (^{1}H NMR, LCMS) and purity (HPLC) analysis and were then assessed for H3N2 and H1N1 activity using the MB-IF assay using A549 cells by Core B. These compounds were also evaluated against H3N2 and H1N1 strains in CPE and virus titer reduction assays using MDCK cells by Research Project sites. Results of these studies are provided in Section G (Core Specific Information). In addition, Core C has also synthesized analogs of influenza polymerase known inhibitor Pimodivir that were designed via computational methods. The design of new analogs was performed via 'CoreHopping' of the indazole and pyrimidine cores of Pimodivir (VX-787) by taking advantage of the published influenza virus polymerase B2/VX-787 co-crystal structure using an 86K and 14K library of compounds, respectively, while maintaining the key interactions. Results of these studies are provided in Section G (Core Specific Information).

B.4 *What opportunities for training and professional development has the project provided?*

We have instituted an undergraduate internship program with University of Alabama at Birmingham Chemistry Department. One undergraduate student worked in Core C and synthesized compounds for the CHIKV program.

C. COMPONENT PRODUCTS

C.1 PUBLICATIONS

Not Applicable

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Not Applicable

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Not Applicable

C.5 OTHER PRODUCTS AND RESOURCE SHARING

Category	Explanation
Research Material	Limited quantities of most synthetic compounds will be made available to qualified individuals for research purposes once the pertinent data has been published. As stated in the original Resource Sharing Plan for Core C (p. 345 of application), and as noted above (C.5.a), once published, and while compound supplies last, we will make research samples of synthetic compounds available to the scientific community for use as chemical probes and for other biological studies. Moreover, we fully expect all biological and chemical data to be published in scientific manuscripts after appropriate patent protection is in place. At this early stage of chemistry, there is no specific resource sharing to report.

D. COMPONENT PARTICIPANTS

Not Applicable

E. COMPONENT IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

Not Applicable

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

NOTHING TO REPORT

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?
Not Applicable

F. COMPONENT CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

Core C was designed to accommodate hit-to-lead phases in all four projects. However, depending on the availability of virus specific assays as well as different stages of success in hit to lead optimization priority changes in the chemistry effort. Hence, some projects are much ahead as compare to others. Our most advanced projects are in alphaviruses followed by DENV and ZIKA. Because of some assay issues in SARS project, medicinal chemistry was slowed down in Year 1. However, now with a fresh plan, the SAR studies will start in Year 2. The HTS data was analyzed from Influenza screen, prioritized chemical series of interest in Year 1. Medicinal chemistry on two series also started this year which will be continued to optimize lead for proof of concept animal model in Year 2. We do not anticipate much challenges in Year 2.

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. COMPONENT SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

Not Applicable

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Not Applicable

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT

Not Applicable

G.10 ESTIMATED UNOBLIGATED BALANCE

Not Applicable

G.11 PROGRAM INCOME

Not Applicable

G.12 F&A COSTS

Not Applicable

RPPR - Core-6074

RESEARCH & RELATED BUDGET - SECTION A & B FINAL

ORGANIZATIONAL DUNS*: 0069005260000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: Southern Research Institute

Start Date*: 03-01-2020 **End Date*:** 02-28-2021

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		Ashish		Pathak		Project Lead	0.00	(b) (6)			29,086.00	12,420.00	41,506.00
2.		(b) (6), (b) (3) (A)				Co-investigator	0.00				17,104.00	7,303.00	24,407.00
3.						Co-investigator	0.00				8,598.00	3,671.00	12,269.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **78,182.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
14	1 Div Proj. Mgr/7 Scientist/6 Chemists	(b) (6)			457,409.00	195,313.00	652,722.00
14	**Total Number Other Personnel**					**Total Other Personnel**	**652,722.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**730,904.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E

ORGANIZATIONAL DUNS*: 0069005260000
Budget Type*: ● Project ❍ Subaward/Consortium
Enter name of Organization: Southern Research Institute

Start Date*: 03-01-2020 **End Date*:** 02-28-2021

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	5,150.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**5,150.00**

E. Participant/Trainee Support Costs		Funds Requested ($)*
1. Tuition/Fees/Health Insurance		0.00
2. Stipends		0.00
3. Travel		0.00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K

ORGANIZATIONAL DUNS*: 0069005260000
Budget Type*: ● Project ○ Subaward/Consortium
Enter name of Organization: Southern Research Institute

Start Date*: 03-01-2020 **End Date*:** 02-28-2021

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	176,948.00
2. Publication Costs	0.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8. Other Charges	40,029.00
Total Other Direct Costs	**216,977.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**953,031.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. OH - Salaries + Benefits	120.0	730,094.00	877,085.00
2. G&A	20.0	1,830,116.00	366,023.00
3. FCCM OH	5.8	730,904.00	42,392.00
4. FCCM G&A (actual rate is 0.10%)	1.0	1,830,116.00	1,830.00
		Total Indirect Costs	**1,287,330.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Darryl Mayes 202-401-2808

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**2,240,361.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification*	File Name: Med Chem Core Budget Justification for Yr2.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Med Chem Core Budget Justification

Key Personnel:

Ashish Pathak, Ph.D. *(Y2: (b) (6) cal. months).* Dr. Pathak will serve as Core Lead for the Medicinal Chemistry Core. Dr. Pathak is Principal Scientist in the Chemistry Department in the Drug Discovery Division of SR and holds an Adjunct Professor appointment in the Chemistry Department at the University of Alabama at Birmingham (UAB). He has over 28 years of experience in medicinal and synthetic organic chemistry, including significant experience in anti-infective and vaccine adjuvant drug design. He has established himself as an independent researcher at Southern Research (SR) and previously led research groups in the Department of Chemistry at Western Illinois University as an Assistant Professor before joining SR. Currently, he manages the high-throughput parallel synthesis group. He is actively involved in research work in drug discovery and is leading medicinal chemist efforts on several externally and in-house funded drug discovery programs. He has extensive experience in all aspects of a medicinal chemistry program, which extends across the spectrum of early lead discovery to lead optimization. He has been PI several NIH-funded programs in the area of viral vaccine adjuvant discovery. He is also leading several drug discovery programs in cancer and anti-infectives. In collaboration with the project PIs and other Co-Investigators in the Center, Dr. Pathak will oversee all aspects of the medicinal chemistry effort, including synthetic target selection, target design, synthesis, and the planning of synthetic routes, compound characterization, structure-activity relationship analysis and interpretation of biological results, cheminformatics and molecular modeling, report, manuscript, and patent application preparation and overall project management of lead optimization chemistry. He will also provide experimental design guidance for ADME and *in vivo* pharmacokinetic (PK) studies.

(b) (6), (b) (3) (A) ***Ph.D.*** *(Y2: (b) (6) cal. months).* (b) (6), (b) (3) (A) will serve as Co-Core Lead for the Medicinal Chemistry and Lead Development Core. (b) (6), (b) (3) (A) is extensively trained as an organic and medicinal chemist, and an accomplished scientific leader with nearly (b) (6), (b) (3) (A) years of drug discovery and management experience. (b) (6), (b) (3) (A) will be responsible for directing all medicinal chemistry and PK efforts for any drug discovery effort of which go/no go decision trees and timelines will facilitate progress and success. (b) (6), (b) (3) (A) has managed more than (b) (6), (b) (3) (A) different drug discovery programs covering at least (b) (6), (b) (3) (A) different therapeutic areas. (b) (6), (b) (3) (A) (b) (6), (b) (3) (A) (b) (6), (b) (3) (A) (b) (6), (b) (3) (A) has been an active member of NIH study sections for several years and has served as a faculty sponsor and mentor to graduate and undergraduate students. (b) (6), (b) (3) (A) has been an advisor to scientific meetings and a reviewer for several journals, (b) (6), (b) (3) (A)

(b) (6), (b) (3) (A) ***Ph.D.*** *(Y2: (b) (6) cal. months).* (b) (6), (b) (3) (A) will also serve as Co-Core Lead for the Medicinal Chemistry and Lead Development Core. (b) (6), (b) (3) (A) He has more than (b) (6), (b) (3) (A) years of drug discovery and management experience, serving in a range of capacities, from bench medicinal chemist to leading drug discovery programs at major pharmaceutical (b) (6), (b) (3) (A) as well as smaller biotech and venture-backed organizations. As a medicinal chemist, he is not restricted to a single therapeutic area and relies on collaborations with fellow investigators for developing novel targets and approaches and for using various methodologies such as HTS-driven or virtual screening to identify hits or leads that can be optimized and advanced into animal models. He and the (b) (6), (b) (3) (A) cumulatively have the capabilities [i.e., assay development, hit identification (screening), and lead development and optimization, utilizing medicinal, computational and analytical chemistry, as well as *in vitro* and *in vivo* PK] needed to support the goal of developing new therapeutics and moving compounds into clinical development.

Other Personnel:

(b) (6), (b) (3) (A) ***M.S., P.M.P.*** *(Y2: (b) (6) cal.).* (b) (6), (b) (3) (A), Senior Manager of Operations for the Drug Discovery Division, has over (b) (6), (b) (3) (A) years of experience in coordinating and managing research projects, and prior to that, (b) (6), (b) (3) (A) where (b) (6), (b) (3) (A) contributed considerably to assay adaptation to high throughput format. (b) (6), (b) (3) (A) will work with Dr. Pathak, the other project leaders and the chemistry team to ensure timely and efficient transfer of project information, resources and data between

internal and external project team members. [redacted] will also track sample transfer between the departments and institutes and manage the testing of samples to assure the deliverables and milestones identified within the project timeline are met.

Medicinal Chemistry Group:

[redacted], ***Ph.D.*** *(Y2: [redacted] cal. months).* [redacted] will support Dr. Pathak in running the Core with his antiviral drug discovery expertise as a Senior Medicinal Chemist. [redacted] He has more than [redacted] years of extensive research experience in the area of synthesis of potential [redacted]. With his broad synthetic background and his experience with [redacted] he has been the lead chemist on several NIH-funded grant projects.

[redacted] ***Ph.D.***, [redacted] ***Ph.D.***, [redacted] ***Ph.D.***, *(Y2: each [redacted] cal. months),* [redacted] ***Ph.D.*** *(Y2: [redacted] cal. months) and* [redacted] ***Ph.D.*** *(Y2: [redacted] cal. months).* [redacted] will provide chemistry services in CETR-funded AD3C Medicinal Chemistry and Lead Development Core. They will be directly responsible for day-to-day synthetic activities in the laboratory. They will perform compound synthesis, compound scale-up, and library research, and will assist in target selection, analog design, synthetic methodology planning, compound characterization, data analyses and cheminformatics. Each of these chemists has several years of research experience in the design and preparation of compounds of many types, and analyzing biological data for structure-activity relationships. They will develop new potent derivatives of the initial lead series with improved ADME properties. Each will devote their efforts in performing hit-to-lead chemistry on active series against various viruses (Projects 1-4) and scale-up synthesis of lead molecules for animal studies.

[redacted] ***M.S.*** *(Y2: [redacted] cal. months),* [redacted] He also has experience in the pharmaceutical industry [redacted] yrs. synthesis and drug design), chemical industry [redacted] yrs. synthesis and route design), and process chemistry [redacted] yrs. synthesis). He will serve as a Chemist for this proposal and will be responsible for the synthesis of compounds and data analysis. He will also be responsible to synthesize intermediates and large scale synthesis.

Bioanalytical Group:

[redacted] ***Ph.D.*** *(Y2: [redacted] cal. months).* [redacted] and will be responsible for all of the *in vitro* and *in vivo* ADME studies that will be used to assess the compounds metabolic stability, solubility and permeability. [redacted] has more than [redacted] years of extensive research, bioanalytical, and [redacted] and in the biotech industry performing studies to support drug discovery and clinical development programs.

[redacted] ***Ph.D.*** *(Y2: [redacted] cal. months).* Under the direction of [redacted], will conduct the *in vitro* ADME studies to determine plasma concentrations of the best compounds. This includes formulation work, dosing and sample analysis. [redacted] has [redacted] years of experience in [redacted], and is skilled in analytical instrumentation and method development. His focus will be to determine plasma concentrations of the best compounds, which includes formulation work, dosing and sample analysis at SR's state of the art bioanalytical laboratory. [redacted] has [redacted] years of experience in [redacted] and is skilled in analytical instrumentation and method development.

[redacted] ***M.S.*** *(Y2: [redacted] cal. months).* Under the direction of [redacted] will be responsible for conducting *in vitro* ADME studies to assess compound metabolic stability and metabolite identification. [redacted] has [redacted] years of experience in activity based proteomics and biochemical assays, as well as [redacted] years of experience utilizing and developing methods for chromatographic and mass spectrometric instrumentation.

Structural Biology and Computational Chemistry Group:

[redacted] ***Ph.D.*** *(Y2: [redacted] cal. months).* [redacted] has over [redacted] years of experience in protein sciences including protein expression, protein purification, functional characterization,

protein identification and structure determination. He holds a [redacted] For this project, [redacted] will provide variable efforts once the viral proteins which the anti-viral compounds are targeting are identified. [redacted] laboratory will provide support such as cloning, expression and purification of target proteins, characterization of protein-compound binding, and determination of protein-compound structures. The goal of [redacted] laboratory in this project is to determine the atomic structure of protein-compound complexes and provide the details to the chemists to assist with the design of potent compounds.

[redacted] ***Ph.D.*** *(Y2: [redacted] cal. months).* [redacted] will provide computational chemistry support including conducting modeling experiments which will be used to prioritize and design compounds for synthesis. In addition, he will assist with developing models and algorithms for structure-activity and PK properties.

Compound Management Group:

[redacted] ***M.S.*** *(Y2: [redacted] cal. months)* [redacted] Chemist, has extensive experience in managing in-house synthesized compounds and commercial libraries. He will provide integrated informatics support including compound tracking, data capture, and data storage, backup, and retrieval. He is in charge of maintaining our in-house [redacted] registration database, which is used extensively in this program to assign a unique identifier to each compound synthesized or commercially-acquired. This identifier number is used throughout the Center to track compounds and any associated data. He is also responsible for sample preparation and distribution to the Assay Core and research project sites.

Other (Non-Labor) Direct Costs *(Y2: $222,127)*

Supplies *(Y2: $176,948).* This budget includes reagents and solvents for synthetic chemist chemists in Y2, estimates based on the historic average cost, catalog pricing from SR-approved vendors (many of whom provide Alabama state contracted rates), historical patterns of laboratory usage, and the PI's past experiences in lead optimization chemistry programs. This budget covers starting materials, specialized reagents, solvents, chromatography supplies, resins and solid-phase synthesis supports, glassware, plastic ware, Eppendorf and other tubes capable of holding larger liquid volumes and other disposables, as well as spectroscopy and compound characterization expenses. It also includes ADME supplies for PK profiling of compounds and potential drug candidates emerging from the lead optimization program. The execution of the various *in vitro* ADME studies, such as solubility, permeability and microsomal stability, requires the purchase of various disposables needed to perform these studies, including microtiter plates, reagents, solvents and other miscellaneous consumables which are essential to the proposed ADME studies. It also includes supplies for the structural biology group to carryout protein purification and crystallization efforts.

Travel Expenses *(Y2: $5,150).* Funds are allocated for the Project Leader and Co-Project Leaders to attend the required NIAID CETR Program Meeting.

Other Charges *(Y2: $40,929).*

In vivo PK Studies *(Y2: $30,000)*. The PK Studies will be out sourced to [redacted]., a Contract Research Organization.

Waste Disposal – *(Y2: $10,029)* has been allocated for the disposal of hazardous chemical and other wastes generated in the execution of the experiments.

A. COMPONENT COVER PAGE

Project Title: Core C.2 Medicinal Chemistry and Lead Development Core - EIDD

Component Project Lead Information:

(b) (6), (b) (3) (A)

B. COMPONENT ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

Primary responsibilities for Core C (MCLDC) of the AD3C include: 1) Hit-to-lead and lead optimization chemistry through structure-activity relationship (SAR) analysis on reconfirmed high-throughput screening (HTS) hits; 2) absorption, distribution, metabolism, and excretion (ADME) and pharmacokinetic (PK) analysis; 3) structural biology; 4) structure-based virtual screening of commercial libraries against relevant X-ray-derived models; 5) analysis of additional HTS data against influenza and Dengue viruses; and 6) purchase or resynthesize promising hits as needed for potential follow-up chemistry, and distribute to the appropriate Research Projects for evaluation in secondary assays. In continuation with the chemistry activities performed in Year 5 of the previous grant, the MCLDC continued doing all of these activities. The Compound Management Group continued to store (-20oC) and distribute all samples to various Research Project sites for additional antiviral analysis using the proper handling and shipping methods for compounds. Compound Management has also been responsible for maintaining the (b) (3) (A), Database in which unique compound identification numbers (SRI Number) are provided and all biological and ADME data for each compound is captured for easy retrieval. The Bioanalytical Group generated all in vitro ADME data which is also captured in (b) (3) (A), system and easily accessible for retrieval and analysis by medicinal chemists. Medicinal chemistry on active HTS hits Dengue virus (DENV), ZIKA Virus (ZIKAV), Severe Acute Respiratory Syndrome (SARS) virus, Chikungunya virus (CHIKV), Venezuelan equine encephalitis virus (VEEV) and Influenza virus] was continued in Yr. 1 of the current AD3C program. Several closely-related analogs were also acquired from commercial sources for reconfirmation of activity and initial SAR activities. All compounds were analyzed for purity by HPLC and integrity by 1H NMR and HR-MS before registering in (b) (3) (A), Synthesis of analogs for each project was prioritized based on the stage of the hit-to-lead optimization and the availability of antiviral assays.
The Specific Aims of Core C, which remain unchanged, are:
Aim 1: Provide lead generation and optimization through medicinal chemistry efforts, SAR studies, structural biology and computational chemistry analyses, and ADME and pharmacokinetic (PK) data to support compound progression into in vivo antiviral efficacy models at Research Project sites. Iterative design-synthesis and testing in SAR assays will be carried out to optimize compounds identified from the current CETR grant. Parameters to optimize will be antiviral potency and efficacy (EC90 < 1 μM; VTR > 3 logs @ 10 μM), cytotoxicity (CC50 > 30 μM) and drug-like properties, such as mouse liver microsomal stability (MLM t½ >60 min) and solubility (>10 μM). Structural biology and computational approaches in drug design will also be utilized, in particular for projects that already have target(s) identified. Nucleoside-based RdRp inhibitors will also be developed along with compounds from previous HTS screens for all viruses. Antiviral activity from a unique collection of 800 nucleosides and nucleoside prodrugs from our partner, EIDD, will also be analyzed. Any hits identified from the Assay Core will go through the iterative design-synthesis process and any compound which meets the antiviral potency and efficacy criteria will be evaluated for in vivo bioavailability and selected for animal studies. Compounds with adequate plasma levels will be tested in vivo. The MCLDC will be responsible for all phases of compound optimization and subsequent synthetic scale-up leading to the in vivo potency studies.
Aim 2: Provide integrated informatics support which includes compound tracking, as well as data capture, analysis, storage, backup and retrieval. Informatics support is an integral part of any drug discovery process, especially for a drug discovery program of this size since a large number of compounds will be synthesized and tested in variety of assays. Careful tracking of compounds submitted to the Assay Core for testing in the primary SAR screen as well as coordination of data generated from the Research Project sites will be important. Compounds coming from Emory Institute for Drug Development (EIDD) will also be distributed to the corresponding Research Project sites via SR's compound management group. SR will use a secured, access-limited, password-protected online file-sharing system (b) (3) (A),) controlled by Southern Research for data sharing and storage for immediate access to collaborators spanning the various Research Project and Core sites.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: 191211_B2 section EIDD report sections by SBM-sm.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

Not Applicable

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

NOTHING TO REPORT

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

Project 1: EIDD supports continued investigation of the in vitro activity, mechanism of action (MOA), and resistance profile of EIDD-2749 against coronaviruses and will supply the material needed to complete all tasks. EIDD will generate and supply EIDD-2749 stability and PK/PD data prior to conducting in vivo models of coronavirus infections.

Project 2: All compounds submitted to projects 1, 3, and 4 have gone to the group for project 2 and works are in progress.

Project 3: EIDD supports continued investigation of the in vitro activity and in vivo efficacy of EIDD-2801 against flaviviruses where appropriate and will supply the material needed to complete all tasks. Currently, EIDD-2801 is being evaluated in a mouse model of Zika virus infection efforts will continue on this initiative. EIDD synthesized and will submit 6 novel ribonucleoside analogs for testing in in vitro models of flavivirus infections. Analogs of actives will be synthesized to improve activity and/or decrease cytotoxicity. Prodrugs will be synthesized to optimize distribution to organs critical in the pathogenesis of flavivirus disease. Plasma and liver microsome stability of synthesized prodrugs will be determined prior to conducting PK/PD studies. EIDD will generate and supply PK/PD data for analogs when appropriate prior to conducting in vivo models of flavivirus infections.

Project 4: EIDD is currently synthesizing analogs of EIDD-2838 to improve activity in the cap-snatching assay. The rate of hydrolysis of EIDD-2838 analogs in aqueous media, liver microsomes and plasma will be determined to establish if stability of the prodrug in the circulating volume is adequate to assure delivery to tissues critical in the pathogenesis of disease. The rate of hydrolysis in cell culture will be determined to establish the relationship between rate of cleavage of the prodrug and efficacy. For the compound EIDD-2801 the current strategy is to file an IND for the treatment of seasonal flu in Q2, 2020 followed by a second IND six months later for the treatment of VEEV infection (Project 3). An expanded access protocol is planned for the treatment of EEEV.

RPPR Report U19 – Core C.2 – MCLD Core EIDD Y1

B.2 What was accomplished in year 1 under the specific aims of the project?

The Emory Institute for Drug Development (EIDD) is an important component of the Antiviral Drug Discovery and Development Center (AD3C) U19 grant. EIDD has a compound library that consist of ribonucleoside and ribonucleotide analogues specifically designed as competitive, alternative substrate inhibitors of virally encoded RNA-dependent RNA polymerases (RdRp). Major activities of EIDD to support all projects are to include the provision of these nucleoside compounds to each group, cell uptake and anabolism, prodrug conjugation, assess PK data. During the current year, the major activities and results are as follows per project:

Project 1: Coronavirus antiviral support by EIDD has led to the finding that EIDD-2749 is a broadly active ribonucleoside analog with activity against Middle East Respiratory Syndrome (MERS-CoV) (EC50 = 5 μM) in a CPE model of infection. The Baric and Denison labs have further confirmed EIDD-2749 activity against MERS (EC50 = 3.47μM) in human airway epithelial cultures.

Project 3: Flavivirus antiviral support by EIDD has led to the finding that EIDD-2801 is a broadly active ribonucleoside analog in late-stage preclinical development to treat seasonal flu and alphavirus infections. 28 day GLP toxicology studies in rats and dogs have been completed.

Project 4: Influenza antiviral support by EIDD has led to the finding that EIDD-2838 is a broadly active ribonucleotide-5'-monophosphate analog with activity against influenza A and B strains (EC_{50} ~1μM) in CPE models of infection. EIDD-2838 was found to be an influenza cap-snatching inhibitor (EC_{50} = 2-4μM) through the AD3C.

C. COMPONENT PRODUCTS

C.1 PUBLICATIONS

Not Applicable

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Not Applicable

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Not Applicable

C.5 OTHER PRODUCTS AND RESOURCE SHARING

Nothing to report

D. COMPONENT PARTICIPANTS

Not Applicable

E. COMPONENT IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

Not Applicable

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

NOTHING TO REPORT

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?
Not Applicable

F. COMPONENT CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. COMPONENT SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

Not Applicable

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Not Applicable

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT

Not Applicable

G.10 ESTIMATED UNOBLIGATED BALANCE

Not Applicable

G.11 PROGRAM INCOME

Not Applicable

G.12 F&A COSTS

Not Applicable

RPPR - Core-6075

RESEARCH & RELATED BUDGET - SECTION A & B **FINAL**

ORGANIZATIONAL DUNS*: 0664699330000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: Emory University

Start Date*: 03-01-2020 **End Date*:** 02-28-2021

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	(b) (6), (b) (3) (A)				Project Lead	(b) (6), (b) (4)	(b) (6)			3,846.00	1,092.00	4,938.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **4,938.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
3	Research Specialists	(b) (6)			86,615.00	24,599.00	111,214.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**111,214.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**116,152.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E

ORGANIZATIONAL DUNS*: 0664699330000
Budget Type*: ● Project ❍ Subaward/Consortium
Enter name of Organization: Emory University

Start Date*: 03-01-2020 **End Date*:** 02-28-2021

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	0.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		Funds Requested ($)*
1. Tuition/Fees/Health Insurance		0.00
2. Stipends		0.00
3. Travel		0.00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K

ORGANIZATIONAL DUNS*: 0664699330000
Budget Type*: ● Project ○ Subaward/Consortium
Enter name of Organization: Emory University

Start Date*: 03-01-2020 **End Date*:** 02-28-2021

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	39,330.00
2. Publication Costs	0.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8. Animal Supplies (mice/rats)	5,823.00
9. Animal per diems (mice/rats)	695.00
10. Maintenance contracts	18,000.00
Total Other Direct Costs	**63,848.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**180,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. F&A	78.5	180,000.00	141,300.00
		Total Indirect Costs	**141,300.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Steven Zuraf 301-492-4855

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**321,300.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification* File Name: Emory Core Budget Justification Yr 2.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

B. BUDGET JUSTIFICATION- BUDGET PERIOD 2

Based on cost of living increases over the past three years at the EIDD, we have projected 3% cost of living increases for personnel. The current fringe rate for Emory University is 28.4%.

(b) (6), (b) (3) (A) **Ph.D.** (sub-Principal Investigator), (b) (6) cal. months/year. (b) (6), (b) (3) (A) has (b) (6), years of experience in the discovery and development of pharmaceutical agents in the biotechnology and large pharmaceutical company sectors. He has been a member of the founding management teams of two highly successful biotechnology companies, (b) (6), (b) (3) (A) (b) (6), (b) (3) (A) held positions of increasing responsibility in the pharmaceutical industry beginning in (b) (6), (b) (3) including (b) (6), (b) (3) (A) He has published over (b) (6), (b) (3) scientific papers and is listed as an inventor on more than (b) (6), patents, (b) (6), of which have led to approved drugs or combinations of drugs for the treatment of HIV and hepatitis B. He has led international drug development teams, which generated data for (b) (6), (b) (3) IND applications and (b) (6), (b) (3) (A) NDAs. (b) (6), (b) (3) (A) He earned a B.S. in chemistry, an M.S. in physical organic chemistry and a Ph.D. in synthetic organic chemistry from (b) (6), (b) (3) (A).

(b) (6), (b) (3) (A) will serve as the Principal Investigator for the Emory subcontract on this proposed program. He will bring to bear his extensive experience for the development of a safe and effective ribonucleoside based therapy for the identification of new antiviral ribonucleosides and ribonucleotides. He will be responsible for overall strategic and scientific oversight as well as coordination of the multidisciplinary drug discovery team that has been assembled for the project.

(b) (6), (b) (3) (A), **PhD,** (Researcher; Nucleoside Chemistry Team Leader) (b) (6) calendar months/year, is an experienced synthetic and medicinal chemist with (b) years of experience as the chemistry team leader for the nucleoside analog program. His training included extensive experience with the synthesis of nucleoside analogs as well as other medicinally active agents to target HCV and drug resistant forms of HIV. He will serve as the chemistry team leader for the duration of this program and he will oversee the day-to-day operation of the chemistry team and coordinate efforts between disciplines.

(b) (6), (b) (3) (A) **Ph.D.** (Postdoctoral Fellow), (b) (6) cal. Months/year is an experienced synthetic chemist who has over a year of experience synthesizing nucleoside and nucleotide based compounds at small and large scale. He has developed unique techniques that are critical for the challenging task of synthesizing highly polar nucleoside compounds and their nucleotide counterparts.

(b) (6), (b) (3) (A), (Molecular Biologist), (b) (6) calendar months/year, (b) (6), (b) (3) (A) with over (b) (6), (b) (3) (A) years of research experience in both the pharmaceutical industry and academia. (b) (6), (b) (3) (A) During (b) (6), time at the EIDD, (b) (6), (b) (3) has also held primary responsibility for the dosing and sampling of small animals for pharmacokinetic and toxicokinetic experiments. In this proposed project, (b) (6), (b) (3) will carry out cellular uptake and anabolism experiments, and provide assistance and supervision in the *in vivo* phases of PK and TK experiments.

.

Supplies

Total consumable supplies and reagents in period 2 = $45,848

Other Costs
$18,000 / year are included in the budget to cover 15% of the maintenance fees for the bioanalytical instruments, which is anticipate being the total proportion of usage of the instruments to cover work for this program.
Costs per year are included to cover the anticipated per diem fees for animal studies.

A. COMPONENT COVER PAGE

Project Title: Project 1.1: The Development of Antivirals for Coronavirus Replication

Component Project Lead Information:

DENISON, MARK R.

B. COMPONENT ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

SPECIFIC AIMS
Zoonotic coronaviruses (CoVs) such as severe acute respiratory syndrome (SARS)-CoV and Middle East respiratory syndrome (MERS)-CoV are pandemic threats, with MERS-CoV continuing to cause new zoonotic and human transmission and illness, resulting in ~35% mortality. Currently, there are no FDA-approved therapies to treat any CoV. Several challenges for anti-CoV drug development exist. 1) As with SARS-CoV and MERSCoV, new zoonotic CoVs likely will emerge from divergent virus pools in animal reservoirs, thus requiring antiviral strategies aimed at conserved targets. 2) Although nucleoside analogues/mutagens are effective against many RNA viruses by acting against viral RNA-dependent RNA polymerases, CoVs uniquely encode a novel RNAdependent proofreading exoribonuclease (nsp14-ExoN), thus requiring effective nucleoside analogues to inhibit or resist the action of ExoN. 3) CoVs are able to rapidly develop resistance to multiple classes of inhibitors, demonstrating the need for approaches to prevent resistance emergence. 4) Both SARS and MERS progress to severe immunopathologic damage, potentially limiting the therapeutic window for direct-acting antivirals (DAAs). Thus, combinations of DAAs and targeted immunomodulators may be necessary for effective treatment of established infection.

The overall goal of our program is to develop CoV antiviral strategies that broadly inhibit known and future potential pandemic zoonotic CoVs, prevent emergence of resistance, and extend the therapeutic window by targeting host immunopathologic responses. Our previous work identified nucleoside analogues (NAs) and non-nucleoside compounds with potent activity against CoVs. Two lead NAs in advanced stages of preclinical development, remdesivir (GS-5734, Gilead Sciences) and EIDD-1931/2801 (Emory Institute for Drug Development), inhibit multiple CoVs, including SARS-CoV and MERS-CoV, in the presence of the intact ExoN. Other promising NAs are also in the pipeline. Two hits from screening at Southern Research (SR), SR-36097 and SR-35293, dramatically inhibit SARS-CoV replication in cell monoculture and in primary human airway epithelial cell cultures. The proposed research will advance preclinical development of the CoV-inhibitory EIDD and SR compounds for treatment and prevention of epidemic and pre-emergent CoVs, generating mechanistic and efficacy data necessary for IND filing and origination of human clinical trials. In addition, we will explore additive or synergistic effects of DAAs. Finally, we will establish proof-of-concept testing for combination of DAAs and immunomodulatory drugs to extend the therapeutic window and treat established infection in vivo. The impact of our program is high. Advancing new leads, increasing potency, broadening activity spectrum, expanding the therapeutic window, and preventing resistance of antiviral treatments for epidemic CoVs addresses an immediate unmet medical need for MERS-CoV and could prove to be an invaluable tool to prevent future pandemics due to pre-emergent CoVs and other high-consequence viral pathogens.

Aim 1. To determine the spectrum of antiviral activity and therapeutic efficacy of EIDD-1931/2801 and perform hit-to-lead stage evaluation of SR compounds. In this aim, we will (i) determine EIDD-1931 and
other DAA activity spectra across CoV groups and genera; (ii) determine antiviral efficacy, metabolism, and cytotoxicity in human lung cells targeted by SARS- and MERS-CoV; (iii) evaluate the efficacy of EIDD-1931 and other DAA NA in young, aged, and immunosuppressed murine models of MERS and SARS pathogenesis; and (iv) perform hit-to-lead studies for SR compounds. SR compounds that meet all Go/No-Go criteria will be entered as lead compounds into the development pipeline as described for EIDD-1931/2801.

Aim 2. To define mechanism of action of EIDD-1931/2801 and impact of resistance mutations on in vivo therapeutic efficacy, viral fitness, and pathogenesis. In this aim, we will (i) define lead compound
mechanisms of action by assessing the impact on CoV replication, chain termination, fidelity, and innate immune responses; (ii) assess the kinetics of drug-resistance development and impact of drug resistance on viral replication and fitness; (iii) determine the effect of drug resistance on sensitivity to other drugs; (iv) determine the impact of resistance mutations on MERS-CoV and SARS-CoV replication, fitness, and pathogenicity in mice; and (v) determine the impact of resistance mutations on therapeutic efficacy. Data collected in Aims 1 and 2 are crucial prerequisites to progression of lead compounds to clinical evaluation.

Aim 3. To test combination regimens for treatment of emerging CoV disease. Emerging coronaviruses evolve quickly and can cause acute severe end-stage immunopathologic lung disease (ARDS), which potentially limits therapeutic windows. Thus, new strategies are needed to extend the effective therapeutic window of DAAs. In this aim, we will (i) assess the combined efficacy and potency of DAAs against MERS-CoV and SARS-CoV in vitro; (ii) evaluate therapeutic and prophylactic efficacy of combined DAAs in murine models of MERS and SARS-induced ARDS; and (iii) determine the therapeutic efficacy of co-administered DAAs and immunomodulators in MERS- and SARS-CoV-infected mice. The impact of these studies is high; investigating new strategies to extend the therapeutic window of DAAs while reversing ARDS disease pathology is broadly relevant to many acute, highly pathogenic respiratory virus infections.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: B2 Project1 CoV AD3C progress report YR1_12-4-19-sm.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

Not Applicable

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

File uploaded: B4 Project1 CoV AD3C progress report YR1_12-4-19-sm.pdf

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

B.6. Plans for the next year.
Our efforts in Year 2 will focus on furthering additional hit compounds through the Go/No Go evaluation scheme. Initial tests will involve potency and activity spectrum. Compounds that meet the Go/No Go criteria described previously will undergo passaging for resistance. In addition, we will continue to determine the mechanism of action and resistance of lead compounds, assess the potential to combine compounds by assessing combined potency and cytotoxicity across a dose matrix, and define the effect of resistance to one drug to efficacy of another drug. We will also further evaluate NHC specificity to viral and not host RNA.

Project 1. The Development of Antivirals for Coronavirus Replication
(Mark R. Denison, (b) (6), (b) (3) (A))

B.2. What was accomplished in year 1 under the goals of the project?

B.2.a. Major activities, specific objectives, significant results, and key outcomes. Our collaborations with (b) (3) (A), (b) (6) Southern Research (SR), and Emory Institute for Drug Development (EIDD) have been productive in the preclinical development of candidate therapeutics against SARS-CoV, MERS-CoV and potentially pre-pandemic zoonotic CoVs. One key candidate was substantially advanced through analytical pipelines of activity spectrum, *in vivo* efficacy, and mechanisms of antiviral activity and viral resistance.

Hit-to-lead generation of new compounds. We continue to work with our collaborators at EIDD, GS, and SR to enter new screening hits into our compound progression pipeline. SR compounds 35020, 36096, 33684, 36100, 42067, 42300, 42301, 41120, 7958, 43551, and 43552 from their medicinal chemistry group did not exhibit antiviral effects in primary human airway epithelial (HAE) cultures. However, two compounds SR-36097 and SR-35293 showed activity against SARS-CoV (>2 log virus titer reduction at 10 μM) in HAEs. Limited analogues of SR-36097 were synthesized to increase potency and enhance pharmacokinetic properties such as metabolic stability. This series was abandoned due to lack of improvement in antiviral potency and narrow intellectual property. An active analogue, SR-35756, of another series, SR-25293, will be tested in HAEs during this upcoming period along with three new SR hit compounds--36899, 35435, 36099, and 35756. In addition, two new hit compounds, EIDD-2749 and EIDD-2838, inhibit MERS-CoV in HAEs with low micromolar EC_{50} values. We will next test the efficacy and potency of these compounds against other divergent CoVs.

Activity spectrum. We previously demonstrated that both remdesivir (GS-5734) and β-D-N_4-hydroxycytidine (NHC; EIDD-1931) are broad-spectrum inhibitors of our panel of endemic, zoonotic, and pre-zoonotic CoVs. In 2016, a new CoV, swine acute diarrhea syndrome (SADS) CoV, was identified as the causative agent of deadly outbreaks of gastroenteritis in pigs in China. Molecular epidemiology and viral metagenomics suggested this new virus likely emerged from bats. Since this virus is genetically related to human CoV 229E, we sought to determine if it was capable of infecting cells from other host species. We constructed a recombinant SADS-CoV expressing a red fluorescent reporter protein (RFP) and found that SADS-RFP virus was able to infect cell lines from pig, human, non-human primate, and cat. Importantly, SADS-CoV was able to productively infect human primary lung microvascular endothelial cells and fibroblasts. Antiviral assays demonstrated that SADS-CoV was susceptible to remdesivir (EC_{50} = 0.02μM). Next steps include determining the efficacy and potency of NHC in inhibiting SADS-CoV replication.

***In vivo* prophylactic and therapeutic efficacy.** To determine the prophylactic and therapeutic efficacy of NHC against highly pathogenic CoVs, we performed studies in our SARS- and MERS-CoV mouse models of pathogenesis. Our dose-finding studies demonstrated that treatment (P.O., B.I.D.) with 50, 150 or 500mg/kg NHC -2hr prior to infection with either SARS- or MERS-CoV was sufficient to abrogate weight loss as compared to vehicle-treated animals. Given that 50 and 150mg/kg treatment failed to completely prevent SARS-CoV replication, we carried the 500mg/kg dose conditions forward in therapeutic treatment studies. Treatment was initiated -2hr, +12hr, +24hr, and +48hr post-infection to explore the therapeutic time window. With SARS-CoV, mice that received treatment initiated at prior to infection and up to 24hr after infection lost less weight, had improved pulmonary function, and had significantly lower lung viral loads as compared to vehicle. Interestingly, mice that were administered NHC beginning 48hr post-infection did not show appreciable clinical improvement yet had significantly reduced titers in the lung. With MERS-CoV, only treatment initiated at or before 12hr post-infection significantly improved weight loss and pulmonary function, although all treatment groups had significantly reduced viral titers (Fig.1.). Using primer ID,

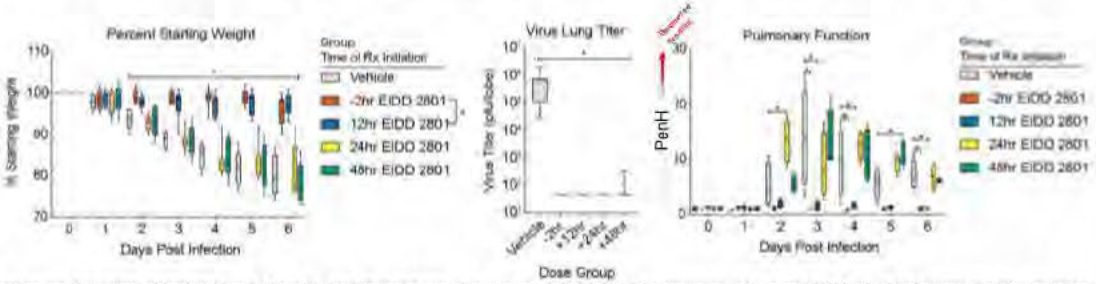


Figure 1. NHC diminishes MERS-CoV disease in mice. 50,000 PFU mouse adapted MERS-CoV was administered intranasally. Mice were treated with 500mg/kg NHC P.O. B.I.D at -2hr, +12hr, +24hr, and +48hr post-infection. Viral titers were reduced in all treatment groups. However, only treatment initiated at or before 12hr post-infection significantly improved weight loss and pulmonary function.

Project 1. The Development of Antivirals for Coronavirus Replication
(Mark R. Denison, (b) (6)

we analyzed both viral RNA and that of a host gene that is significantly upregulated following MERS-CoV infection (ISG15) to determine if NHC would selectively mutagenize viral RNA in mice. We observed a dose-dependent increase in mutation frequency of viral RNA, but not in the host mRNA ISG15. We also noted an increase in the viral nucleotide transition rate, which was consistent with our cell culture observation. NHC-induced mutations resulted in codon changes, including premature stop codons, in viral RNA. Thus, NHC-mediated mutagenesis was directly correlated with protective efficacy *in vivo*.

Mechanism of action. Our previous data showed that MHV and MERS-CoV acquire a large number of mutations at the consensus level when passaged in the presence of NHC. To probe this phenotype further, we treated WT MHV with increasing concentrations of NHC and performed full-genome next-generation sequencing (NGS) on viral populations released after a single round of infection. The results demonstrated a dose-dependent increase in mutations across the genome. The majority of mutations were G:A and C:U transitions. A dose-dependent increase in C:U mutations was also detected in MERS-CoV genomes produced during treatment of HAEs with a range of NHC doses. When we determined the ratio of infectious virions per viral genome (specific infectivity) released from MHV-infected monolayers treated with NHC, we observed a dose-dependent decrease in specific infectivity. This result indicated that NHC treatment reduces the fraction of infection-competent progeny genomes. To determine if this outcome was attributable to an increased production of defective viral genomes, we quantified genome recombination frequencies at each NHC concentration using a specialized bioinformatics pipeline. Recombination frequency did not change in the presence of NHC, indicating that this compound does not increase defective genome formation. These data further strengthen our evidence that the primary mechanism of NHC anti-CoV activity involves lethal mutagenesis of viral RNA, consistent with NHC mechanism of action described for other viruses, including influenza, IBV, VEEV, and RSV.

Identification of NHC resistance mutations. We passaged MHV and MERS-CoV in NHC-treated cultured cells to assess viral capacity to acquire resistance and gain insights into viral targets of NHC action. By passage 30, one lineage of MHV and two lineages of MERS-CoV displayed low-level (~2-fold) resistance to NHC. Whole-genome sequence analysis revealed a high mutation frequency, with 102 non-synonymous mutations present in P30 MHV and 27 and 41 non-synonymous changes acquired in P30 MERS-CoV. To identify which mutations conferred resistance, we began by engineering mutations in likely targets of NHC, replicase genes, into WT MERS-CoV. These included a V558I change in nsp12, which encodes the RNA-dependent RNA polymerase (RdRp), and two changes in the N7-methyltransferase domain of nsp14. Preliminary data suggest that the triple mutant is less sensitive to NHC compared to WT. Next steps include confirming the phenotype and comparing fitness of NHC-resistant strains to WT MERS-CoV.

Impact of drug resistance on the viral susceptibility spectrum. To assess reciprocal effects of remdesivir and NHC resistance, we treated remdesivir-resistant MHV with increasing doses of NHC and NHC-resistant MHV with increasing doses of remdesivir and quantified viral replication. Our results suggest that remdesivir-resistant MHV is more sensitive to NHC than WT virus, whereas NHC-resistant MHV is equally or marginally more sensitive to remdesivir than WT virus. NHC-resistant MERS-CoV is more sensitive to remdesivir than WT MERS-CoV. Based on these findings, we hypothesize that combination treatment with these compounds may impede resistance development in CoVs.

Combination therapy. We determined whether NHC and remdesivir used in combination alters anti-CoV potency. MHV-infected cells were treated with a dose matrix of NHC and remdesivir in which concentrations of the two compounds were co-varied. A CellTiterGlo cell-viability assay optimized for high-throughput screening was used to determine the effect of drug combinations cytotoxicity and on the cytopathic effect of MHV replication. The results reveal a positive interaction between NHC and remdesivir in the inhibition of MHV replication, while cytotoxicity remained below acceptable levels. These results, in conjunction with absence of detectable cross-resistance, hold promise that NHC and remdesivir could be amendable to use as combination therapy against CoVs.

Project 1. The Development of Antivirals for Coronavirus Replication
(Mark R. Denison, (b) (6))

B.4. Training Opportunities.
Graduate students are active in the project at Vanderbilt and UNC. Individual development plans (IDPs) are generated on an annual basis for all graduate students. These are used for defining key objectives and goals and reviewed on at least an annual basis. For the AD3C program, IDPs include specific goals relevant to the project. IDPs assist in analysis of progress and future training and career development. Construction of the IDP includes creation, review, and updating of biosketches and CVs; these serve as learning tools for presenting professional training and accomplishments in formats relevant to research funding proposals.

C. COMPONENT PRODUCTS

C.1 PUBLICATIONS

Not Applicable

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Not Applicable

C.3 TECHNOLOGIES OR TECHNIQUES

Category	Explanation
Research Material	We constructed a recombinant SADS-CoV expressing a red fluorescent reporter protein (RFP). This expands our panel of pre-zoonotic CoVs used for drug activity spectrum determination.

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Not Applicable

C.5 OTHER PRODUCTS AND RESOURCE SHARING

Nothing to report

D. COMPONENT PARTICIPANTS

Not Applicable

E. COMPONENT IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

Not Applicable

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

NOTHING TO REPORT

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?
Not Applicable

F. COMPONENT CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. COMPONENT SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

Not Applicable

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Not Applicable

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT

Not Applicable

G.10 ESTIMATED UNOBLIGATED BALANCE

Not Applicable

G.11 PROGRAM INCOME

Not Applicable

G.12 F&A COSTS

Not Applicable

RESEARCH & RELATED BUDGET - SECTION A & B FINAL

ORGANIZATIONAL DUNS*: 0799178970000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: Vanderbilt University Medical Center

Start Date*: 03-01-2020 **End Date*:** 02-28-2021

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr	Mark		Denison	MD	Site Principal Investigator	0.00	(b) (6)			28,845.00	3,663.00	32,508.00
2.		(b) (6), (b) (3) (A)			MD	Co-Investigator	0.00				13,105.00	2,844.00	15,949.00
3.					PhD	Co-Investigator	0.00				45,735.00	9,924.00	55,659.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **104,116.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
3	2 Sr. Research Specialists, 1 Research Asst.	(b) (6)			69,477.00	18,480.00	87,957.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**87,957.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**192,073.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E

ORGANIZATIONAL DUNS*: 0799178970000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: Vanderbilt University Medical Center

Start Date*: 03-01-2020 **End Date*:** 02-28-2021

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	6,000.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**6,000.00**

E. Participant/Trainee Support Costs		Funds Requested ($)*
1. Tuition/Fees/Health Insurance		0.00
2. Stipends		0.00
3. Travel		0.00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K

ORGANIZATIONAL DUNS*: 0799178970000
Budget Type*: ● Project ○ Subaward/Consortium
Enter name of Organization: Vanderbilt University Medical Center

Start Date*: 03-01-2020 **End Date*:** 02-28-2021

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	53,538.00
2. Publication Costs	3,500.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8. Shipping costs	600.00
9. Sange Sequencing and Deep Sequencing	24,150.00
10. Repairs and Maintenance	10,500.00
Total Other Direct Costs	**92,288.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**290,361.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. Modified total direct cost base	58.0	290,361.00	168,409.00
		Total Indirect Costs	**168,409.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number)	Department of Health and Human Services (DHHS) Steven Zuraf (301) 492-4855

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**458,770.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification*	File Name: U19- Budget Justification YR2_12-3-19.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

VUMC BUDGET JUSTIFICATION (PROJECT 1-[redacted])

PERSONNEL

Mark R. Denison, M.D. (Site PI, [redacted] effort, [redacted] calendar months). Dr. Denison will serve as the Principal Investigator of Project 2. Dr. Denison has over 30 years of experience studying coronavirus replication and replicase nonstructural protein functions. He has published extensively on replication, molecular biology, protein functions and fidelity of coronaviruses. He has a long-standing, highly productive collaboration with [redacted] for joint studies of the pathogenesis and inhibition of SARS-CoV and MERS-CoV, with strong synergy of directed mutagenesis and *in vitro* replication and biochemistry studies in the Denison lab and *in vivo* pathogenesis studies in the [redacted] Dr. Denison and [redacted] communicate directly on a weekly basis on this project. Dr. Denison will oversee these collaborations and will schedule conference calls and meetings. [redacted] labs meet in person three times a year and have monthly phone calls for discussion of collaborative projects).

[redacted] **Ph.D. (Co-Investigator, [redacted] effort, [redacted] calendar months).** [redacted] joined the [redacted]. [redacted] has [redacted] years of virology experience at BSL-2 and will carry a leading role in the laboratory antivirals program. Prior to joining the lab, [redacted] will oversee and perform studies in Aims 1, 2, and 3. [redacted] will be a BSL-3 / Select Agent investigator and will work with [redacted] to perform studies using MERS-CoV and SARS-CoV at BSL-3. Additionally, [redacted] will interact with collaborators on data analysis, presentation, and publication. [redacted] will serve as the organizer of scientific data and discussions from the group, and interact with Program Managers at UNC, UAB, Emory, and Gilead.

[redacted], **M.D., Ph.D. (Co-Investigator, [redacted] effort, [redacted] calendar months).** [redacted] He directs the Vanderbilt BSL-3 SARS-CoV and MERS-CoV research program. [redacted] is an authorized Select Agent user and will coordinate all investigators and studies in the BSL-3. He will oversee all experiments using engineered SARS-CoV and MERS-CoV in the BSL-3 and contribute advice on scientific direction and experimental strategy.

[redacted] **M.S. (Senior Research Specialist, [redacted] effort, [redacted] calendar months).** [redacted] has worked with Dr. Denison for over [redacted] years. [redacted] will support experiments at BSL-2 for Aim 2, including preparation of mutagenized reverse-genetic plasmids, viral RNA sequencing, and data analysis. [redacted] will coordinate all project purchases.

[redacted] **M.S. (Senior Research Specialist, [redacted] effort, [redacted] calendar months).** [redacted] joined the [redacted] lab in [redacted]. [redacted]. [redacted] also will work with [redacted] to perform experiments to determine the combined effect of multiple drugs on MHV replication. [redacted] will coordinate all project purchases.

[redacted] **M.S. (Research Assistant III, [redacted] effort, [redacted] calendar months).** [redacted] joined the [redacted] lab in [redacted] Prior to joining the lab, [redacted] gained previous experience performing cell culture and qPCR experiments in a research setting. [redacted] is currently training for approval as a Select Agent user and will work with [redacted] and [redacted] to passage MERS-CoV and SARS-CoV in the presence of drugs, identify resistance mutations, and perform quantitative testing of CoV-active lead compounds and structural analogues at BSL-3.

FRINGE BENEFITS: Fringe benefit calculations are derived from the current Vanderbilt University Medical Center guidelines.

LAB SUPPLIES (Year 2: $53,538)

Cell Culture Supplies (Year 2: $11,738). Funds are requested for media, serum, sterile plastic ware, and related cell culture supplies to maintain multiple cell lines and measure the effect of drugs on a variety of virus replication parameters by plaque assay and plate reader-based assays. We predict an increase in cell culture supply cost in years 2-5 based on the scope of work and the experimental timeline.

BSL-3 Supplies (Year 2: $16,000). All testing of drugs against SARS-CoV and MERS-CoV, including virus passaging, monitoring development of drug resistance, and confirmation of resistance to lead compounds, will be performed under strict BSL-3 protocols. This will include extensive use of plastic ware, tissue culture reagents, and materials for plaque assays and RNA isolation. BSL-3 PPE (personal protective equipment) is required for all work done at BSL-3. Materials for autoclaving and disposal of waste are also included in this category. Dedicated reagents are required for analysis of SARS-CoV RNA at BSL-2 because viral RNA is regulated as a Select Agent. We predict an increase in BSL-3 supply cost in years 2-5 based on the type and number of MERS- and SARS-CoV experiments proposed in those years.

Enzymes and Reagents (Year 2: $8,400). Engineering mutations within plasmids carrying fragments of viral genomes for the experiments proposed in Aims 2 and 3 will require enzymes and reagents specified by the corresponding protocols. Assembling recombinant MHV, SARS-CoV, and MERS-CoV requires large amounts of expensive restriction enzymes (e.g., BsmBI) and DNA ligase. Molecular biology grade agarose, DNA purification kits, and DNA markers are needed for identifying and isolating assembly intermediates to generate full-length cDNA clones of recombinant viruses. In addition, recovery of viruses from cloned cDNAs requires expensive T7 RNA polymerase-driven *in vitro* transcription kits and electroporation supplies for introduction of infectious full-length viral RNA transcripts into susceptible cells. Substantial amounts of real-time PCR supplies and reagents (e.g., reaction kits and fluorescent probes) are required to support experimental procedures proposed in Aims 2 and 3. Cytotoxicity kits (e.g., CellTiter-Glo) as well as the 96-well plates and other plastic ware will be used to examine the toxicity of drug combinations in Aim 3. Basic chemicals and buffers are needed for general laboratory techniques.

MinION sequencing (Years 2: 16,000). Experiments proposed in Aim 2 require specialized reagents for MinION library preparation and long-read single-molecule sequencing ($830/sample; estimated 34 samples/year).

CO2 and LN2 (Year 2: $700). Cell culture requires regular delivery of CO_2 for incubators, and cryopreservation of cell lines used in this project requires periodic deliveries of LN_2. We predict an increase in CO2 and LN@ cost in years 2-5 based on an increase in incubator and cryopreservation capacity needs as the experimental timeline progresses.

Computer supplies (Year 2: $700). Funds are requested for project specific computer and software upgrades required to analyze data and communicate results.

OTHER EXPENSES (Year 2: $44,750)

Sanger sequencing (Year 2: $1,750). Funds are requested for Di-deoxy (b) (6), (b) (3) (A) sequencing of full viral genomes to identify mutations in Aim 2 and to confirm engineered mutations in genome fragments and potential reversion and compensating mutations in Aims 2 and 3.

Deep sequencing (Years 2: 22,400). Funds are requested towards deep sequencing of viral genomes to determine mutation rates and types induced by drug treatment. Cost of deep sequencing for year 1 is budgeted at 32 samples at $700/sample which includes amplicon construction by our laboratory followed by and library preparation and sequencing by the (b) (6), (b) (3) (A) core. We predict a decrease in deep sequencing cost in years 2-5 based on the experimental timeline.

FedEx Shipping (Year 2: $600). Funds are requested to cover the costs of shipping samples to the (b) (6), (b) (3) laboratory for analysis. We predict an increase in shipping cost in years 2-5 based on the expected increase in materials produced by each laboratory.

Repairs and Maintenance (Year 2: $10,500): This category includes maintenance of equipment and service contracts on major equipment in BSL-2 spaces as well as annual decontamination and complete recertification of the BSL-3 lab, which includes required preventative maintenance of equipment and service contracts on major equipment. The BSL3 decontamination and recertification includes replacement of HEPA filters, PM, and vapor phase H_2O_2 sterilization. The percent of cost for this proposal for each year is based on the number of ongoing BSL-3 grants / projects. At this time we propose 33% of cost for this process attributable to this proposal.

Publications (Year 2: $3,500): Sufficient for 1 publication per year. This is increased in out years as more publications are anticipated.

TRAVEL (Year 2: $6,000): Budgeted amount will allow partial support of travel for two investigators to attend one meetings per year for presentation of scientific results. Relevant venues include the International Congress on Antiviral Research (ICAR), and the American Society for Virology. In addition, these funds will allow travel for participating investigators to the University of Alabama at Birmingham (UAB) for the annual Centers for Excellence in Translational Research (CETR) symposium at UAB. This is increased in years 2-5 based on the potential international location of the nidovirus and ICAR meetings.

A. COMPONENT COVER PAGE

Project Title: Project 2.1: Novel Therapeutics for Emerging Alphavirus
Component Project Lead Information: STREBLOW, DANIEL N

B. COMPONENT ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

SPECIFIC AIMS
The goal of this project is the continued development of novel antiviral drugs against emerging alphaviruses. Alphaviruses are arthropod-transmitted RNA viruses comprised of multiple NIAID Category B and C priority pathogens including Venezuelan equine encephalitis (VEEV) and chikungunya (CHIKV) viruses, as well as other re-emerging viruses such as Eastern equine encephalitis (EEEV), Mayaro (MAYV), o'nyong-nyong (ONNV), and Ross River (RRV) viruses. The encephalitic alphaviruses primarily present with neurological disease that can result in high mortality rates in hosts with weakened or immature immune systems, including young and aged populations. Arthritogenic alphaviruses are associated with incapacitating acute and chronic arthralgia, arthritis, and tenosynovitis that can result in long-term disability, and large epidemics with severe economic consequences. Currently, no FDA-licensed vaccines or antiviral therapeutics are available to prevent alphavirus infection or treat disease and thus a critical unmet clinical need exists to combat these viral agents.
In collaboration with Southern Research (SR), we performed high-throughput screen (HTS) campaigns, and confirmatory secondary assays, to identify small molecules that inhibit VEEV and/or CHIKV replication. These screening efforts resulted in the identification of a number of non-toxic lead compounds that effectively block the replication of VEEV, CHIKV, and other alphaviruses. Furthermore, we generated resistant mutants against two highly active compounds and, in doing so, identified the alphavirus nsP2 helicase domain and the nsP3 macrodomain as potential antiviral targets. In addition to this work, collaboration with the Emory Institute for Drug Development (EIDD) identified novel nucleosides, which target RNA-dependent RNA polymerases, with antiviral activity against alphavirus infection in vitro and in vivo. Thus, we have identified potent antiviral compounds against three distinct molecular targets of alphavirus replication.
In this CETR application, we plan to (i) further chemically optimize the lead compounds for antiviral potency, efficacy, and drug-like properties, (ii) define the phylogenetic breadth of antiviral activity, mechanisms of action, and synergism of lead compounds, and (iii) evaluate antiviral activity with single agents and in combination with other leads identified in this project. In addition, we will explore combinations of these antiviral compounds with anti-inflammatory therapies against alphavirus infection in well-characterized pre-clinical animal models of acute and chronic alphavirus disease. To develop novel inhibitors that block alphavirus replication, prevent the emergence of resistance viruses, and reduce disease severity, we propose the following Specific Aims:
Specific Aim 1. To further optimize the inhibitors of alphavirus replication to improve potency and bioavailability. Our previous work resulted in the identification of four chemically distinct leads (SRI-33001, SRI-33394, SRI-34626, and SRI-34963). These leads display potent antiviral activity against diverse alphaviruses with low cytotoxicity. Furthermore, we identified novel nucleoside inhibitors of alphavirus replication, including the lead compound EIDD-1931 and prodrug (EIDD-2801), as well as an active 4'fluorouridine analog EIDD-2749. In this Aim, these lead compounds will be optimized by iterative medicinal chemistry, cell culture based antiviral and cytotoxicity assays, and in vivo pharmacokinetic studies to improve their efficacy, selectivity, solubility, and bioavailability. Optimized compounds will advance to mechanistic (SA2) and pivotal efficacy (SA3) studies.
Specific Aim 2. To determine the antiviral mechanisms of inhibitors of alphavirus replication. In this Aim, we will define the breadth of antiviral activity and cell type specificity, determine the molecular targets through resistance mapping and structural/computational analyses, and identify synergy profiles for active compounds. In preliminary studies, characterization of resistant mutants derived against two highly inhibitory compounds identified the alphavirus nsP2 helicase domain and the nsP3 macrodomain as likely molecular targets. To explore the activity of compounds against these specific viral targets, we have generated a cell line expressing nsP2 helicase and purified nsP2 protein for biochemical/structural assays. Furthermore, we have generated a 1.46 Å resolution crystal structure of the nsP3 macrodomain. We will use this new structural information for in silico computational modeling and empirical structural biology analysis to identify regions of the macrodomain targeted by small molecule inhibitors and their analogs. Finally, we will define the mechanisms of action for our anti-alphavirus compounds and structurally related active analogs that are yet uncharacterized. These studies will inform our compound selections for synergy studies in the context of combination therapy (SA3).
Specific Aim 3. To define the efficacy of mono and combination therapies against alphavirus replication and disease. In this Aim, compounds with optimized antiviral and pharmacokinetic profiles will be evaluated using well-established pre-clinical murine models of alphavirus infection and disease. Given that selection for antiviral drug resistance can adversely impact therapeutic efficacy, and that combination therapies targeting two or more regions of the virus should generate a higher barrier to resistance, new therapeutic regimens will be tested that combine our small molecule inhibitors with novel nucleoside analogs that show robust in vivo efficacy. Lastly, since alphavirus disease is caused in part by virus-induced inflammatory immune pathology, we will evaluate the therapeutic potential of antiviral therapy combined with anti-inflammatory regimens to block both virus replication and virus-induced immune pathology.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: B.2 only Accomplishments Proj 2 2019.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

Not Applicable

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

File uploaded: B.4 Training Opportunities Proj 2.pdf

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

B.6 Plans for next year:
Overview: The goal of this project is to identify novel small molecules capable of inhibiting the replication of diverse members of the Alphavirus genus. We have identified a number of hit molecules with antiviral activity that are at different stages of development. Below is a brief discussion pertaining to each of the compounds that are active in our program and the goals for the upcoming year.
Quinolinones (SR-33394): We are in the process of assembling a manuscript to describe the results from the medicinal chemistry campaign aimed at identifying SAR around this chemical series. Our immediate goals are to perform complementary experiments to identify the breadth of cell types for which SR-33394 compounds are active. We will then finish up writing and submit a manuscript describing these results.
Benzoannulenes (SR-33366): Our immediate goals for this year for this compound series is completion and submission of a manuscript that defines this class of compounds as DHODH inhibitors with activity against a number of RNA viruses including alphaviruses and flaviviruses. In addition, we will perform studies aimed at determining whether combination therapy of this class of inhibitors with other inhibitors including nucleoside analogs will result in antiviral synergism. We hypothesize that combination of DHODH inhibitors with nucleoside analogs may push virus replication into crisis because we will be blocking de novo pyrimidine synthesis and forcing the virus to use the mutating or chain terminating nucleosides. This should enhance the activity of the nucleosides. IF this works then we will try the combination in vivo.

SR-36426: Our immediate goals with this compound series is to continue with the ongoing medicinal chemistry campaign to gain critical information about SAR, to improve solubility and maintain or enhance antiviral activity. We will continue to screen novel compounds based upon this series and attempt to establish a resistant phenotype in order to identify potential mechanisms of action for this series.

SR-33001. Medicinal chemistry will continue to be performed with this compound series to identify compounds with improved pharmacological properties. While an in vivo experiment showed about a two-log reduction in viral loads, this was performed with a compound with solubility issues that were even more apparent in vivo. In addition, at the end of the past year, SR was able to synthesize a biotinylated analog for this series. During the next year we plan to use this compound as a probe to identify the drug target. Thus, our immediate goals are to improve solubility and identify the molecular target(s) for this very interesting chemical series.

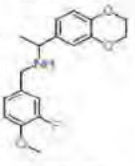
SR-36767. This class of compounds is a first-in-class for CHIKV in that it appears to be a non-nucleoside small molecule inhibitor of nsP4 (viral RNA dependent RNA polymerase). This compound has good antiviral activity in mice. We will continue to perform in vivo efficacy studies for this SR-42718. Immediate goals include performing an experiment to assess whether a higher dosage (80mg/kg; TID) will be sufficient to further reduce tissue viral loads at 7 and 10 days post challenge. Then experiments will be performed that will determine the effect that SR-42718 has on joint pathology as well as to determine the effect of treating at 1 day post infection. The in vivo mouse experiments for SR-42718 should be finished in Year 2. We hope to procure follow-on funding to perform in vivo efficacy experiments in nonhuman primates. In addition, we plan to fully characterize the resistance phenotype that we observed and to increase the breadth of antiviral activity against additional alphaviruses through continued medicinal chemistry studies. We will also initiate structure function studies in order to fully explore nsP4 as a target of this series in order to develop additional small molecules that target nsP4.

Additional Compounds. We validated three compounds including SR-7958, SR-8380, and SR-18457 against a number of alphaviruses. We plan to synthesize and characterize additional nucleoside analogs based upon these scaffolds in order to improve their activity and bioavailability. We will also continue to characterize their molecular action and attempt to generate resistance. Compounds with appropriate activity and bioavailability profiles will be tested in the last part of this upcoming year for efficacy against CHIKV infection in mice.

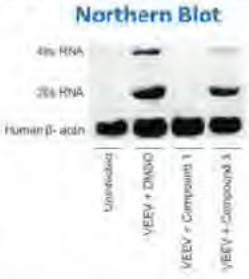
B.2 The goal of this project is to identify novel small molecules capable of inhibiting the replication of diverse members of the Alphavirus genus. Alphaviruses are arthropod-transmitted RNA viruses comprising seven antigenic complexes that include multiple Biodefense Category B and C priority pathogens. Currently, no FDA-licensed vaccines or antiviral therapeutics are available to prevent alphavirus infection or treat disease and thus a critical unmet clinical need exists to combat these viral agents. In collaboration with SR, we performed new high-throughput screen (HTS) campaigns, and confirmatory secondary assays, to identify additional small molecules that inhibit VEEV and/or CHIKV replication. These screening efforts resulted in the identification of a number of non-toxic lead compounds that effectively block the replication of VEEV, CHIKV, and other alphaviruses. During the previous grant period, medicinal chemistry was used to identify more potent, soluble, and stable compounds. Our aim is to chemically optimize alphavirus inhibitors, determine their mechanism of action and breadth and then evaluate the in vivo efficacy of lead compounds. This report summarizes our efforts in 2019 to develop small molecules inhibitors of alphaviruses.

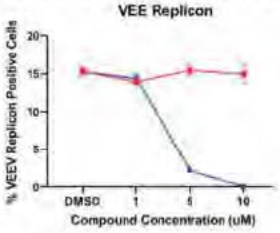
Analysis of compounds from the 2015 VEEV HTS campaign. SR screened 197,025 compounds in a VEEV CPE-based assay in Vero E6 cells and identified 23 compounds for immediate follow-up. OHSU confirmed 8 of 12 active hits including: SR-36426 (IC_{90}=0.72μM), and SR-36427 (IC_{90}=0.25μM).

SRI-36427

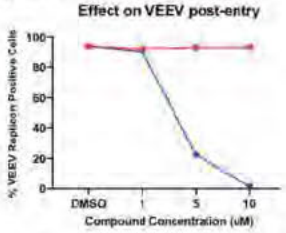


SR-36427. SR generated 59 SR-36427 analogs, but none dramatically improved activity profiles. Analogs SR-38669 (IC_{90}=0.38μM; VLR=9.1 logs at 10μM) and SR-39023 (IC_{90}=0.37μM; VLR=4.88 logs at 10μM) were promising but still had issues of solubility and/or microsomal stability (MLM $t_{1/2}$ = 1.5 minutes). VEEV RNA (northern blot analysis) and protein synthesis were inhibited by SR-36427 indicating that the drug targets nonstructural protein (nsP) functions. SR-36427 blocks VEE replicon activity following infection or transfection, indicating that the compound targets virus replication at a post-entry stage. The generation of resistance was attempted but failed.





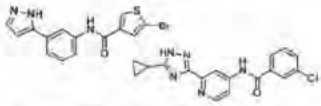
Infection with Replicon



Transfection of Replicon

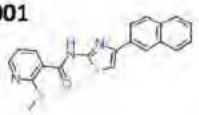
Based upon the inability to generate active analogs with improved stability, SR-36427 has been put on hold. A manuscript describing antiviral activity for the SR-36427 series was accepted for publication in ACS-ID.

SRI-36426 **SRI-42776**



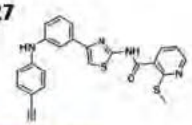
SR-36426. SR generated 75 analogs of SR-36426. The compound displays high activity and low toxicity profiles and some SAR promising analogs display improved activity and solubility. SR-36426 is broadly active against 5 different alphaviruses. SR-36426 inhibits vRNA synthesis and blocks structural protein translation. SR-36426 effectively blocked virus replication in infectious viral RNA transfected cells. *In vivo* pK analysis for SR-42776, a SR-36426 analog, indicated a fast clearance rate ($t_{1/2}$=2.2 hours). SR will continue to synthesize additional analogs for this series to improve pK properties and *in vivo* experiments will be performed on any lead compounds for this series.

SRI-33001

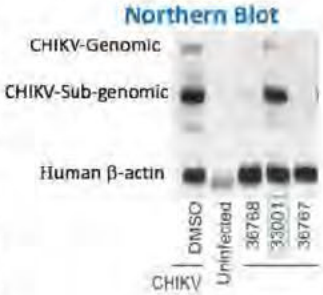


Analysis of compounds from the 2015 CHIKV HTS campaign. SR screened 197,025 compounds in a CHIKV CPE-based assay in Vero E6 cells and identified 26 compounds for immediate follow-up. We confirmed 5 of 11 hits including: SR-33001 (IC_{90}=0.93μM), SR-35756 (IC_{90}=3.39μM), SR-35894 (IC_{90}=0.75μM), and SR-36767 (IC_{90}=0.09μM).

SRI-41627

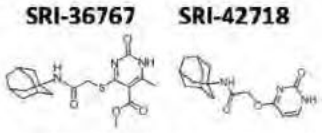


SR-33001. SR generated 90 SR-33001 analogs. SR-41627 (CHIKV-IC_{90}=0.45μM; VLR=8.68 logs at 10μM) was tested for *in vivo* pK activity and found to be devoid of overt toxicity and the compound was cleared with a $t_{1/2}$=6.5hr. SR-33001 is broadly active against alphaviruses, flaviviruses and human cytomegalovirus. SR-33001 does not block viral RNA or nsP protein synthesis but efficiently blocks structural protein synthesis. SR-33001 synergizes with compounds that block RNA synthesis. This profile is similar to our observations with the Src kinase inhibitor Dasatinib (Broeckel *et al.* 2019), which blocks phosphorylation of the kinase S6. SR tested the SR-33001 analog SR-41627 against

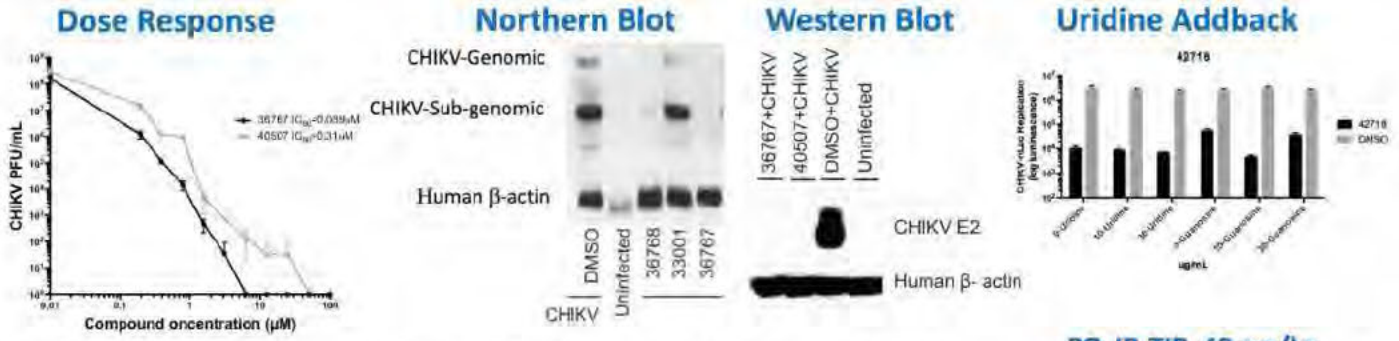




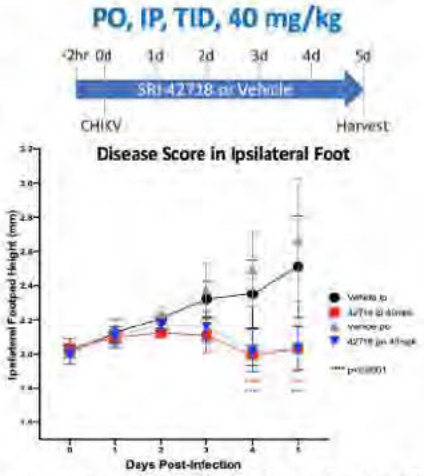
a panel of kinases and OHSU tested SR-33001 for down-regulation of phosphorylation of S6. While Das blocked phosphorylation of S6 in CHIKV-infected cells, this was not observed in cells treated with SR-33001. Collectively, these data suggest that SR-33001 blocks translation of the CHIKV subgenomic vRNA at a step that is different from Dasatinib. Despite extensive attempts, we have been unable to select for resistance against this compound suggestive of a cellular target or an essential viral target that is common among very divergent viruses. In addition, SR-33001 was screened against a limited panel of kinases and no inhibitory activity was observed. In vivo efficacy studies showed little activity, which may be due to poor solubility or permeability for SR-41627. A biotinylated probe has been synthesized for SR-41627 to facilitate target identification studies. Further SAR is aimed at improving solubility, microsomal stability and *in vivo* pK.



SR-36767. SR generated 50 SR-36767 analogs. SR-36767 (IC_{90}=0.089μM; VLR>7 logs at 10μM) is active against many alphaviruses but not flaviviruses. SR-40507 is a pyrimidone analog (IC_{90}=0.31μM; VLR=7.44 logs at 10μM) that was used as a new scaffold to generate SR-42718 (IC_{90}=0.09μM; VLR>7 logs at 10μM), which was recently tested for *in vivo* pK ($t_{1/2}$=6.59hr). The SR-36767 series blocks viral RNA expression (northern blot analysis) and reduces structural protein levels (western blot analysis). DHODH inhibition assays and uridine add-back experiments indicated that the compound was does not target this biosynthetic pathway. Resistance was derived against the analog



SR-40507, which tracks with a mutation in nsP4 (RdRp). Resistance derived against SR-40507 was also active against SR-36767 and SR-42718. Subsequent cloning of the single G to A mutation (Alanine to Threonine) into an infectious clone of CHIKV provided resistance. Preliminary modeling indicates that the mutation is positoned at the inner leaflet of the RNA template tunnel. SR-42718 has a solubility of 62μM in water and decent microsomal stability in mouse, monkey and human microsomes. The compounds is inactive by MiniAmes testing and Cyp activity assays. *In vivo* PK analysis indicates that the compound has good bioavailability by both PO and IP delivery mechanisms but the compound has a short $t_{1/2}$. No adverse toxicity was found by preliminary toxicity studies in mice and the compound was distributed to a number of tissue types including joints and muscles. Importantly, treatment of mice with SR-42718 at 40mg/kg (3x daily; i.p. or p.o.) was sufficient to completely block CHIKV viremia at 3 dpi, reduce joint viral loads by 2-logs at 5 dpi, and reduce joint swelling (from 2-10 dpi) in a mouse model following footpad challenge. BID studies were met with reduced efficacy indicating that TID was required for full activity. In both a 7 & 10 day treatment regimen virus was detected in the ipsilateral joints to near vehicle control levels indicating that either the virus may develop resistance or compound levels dropped below the effective level. Further studies are being designed to improve *in vivo* delivery, bioavailability and efficacy testing. Further SAR is focused on developing compounds with improved pK activity as well as increased breadth of antiviral activity.



Nucleoside Compounds: We validated three nucleoside compounds including SR-7958, SR-8380, and SR-18457 against a number of alphaviruses. SR-7958 and SR-18547 are broadly active against CHIKV, MAYV, ONNV, UNAV, RRV and VEEV. SR-8380 had better activity against CHIKV and UNAV than the other four viruses tested. Time of compound addition studies with CHIKV indicate that all of these compounds must be delivered by 4 hpi in order to achieve antiviral activity. The compounds block viral RNA expression by Northern blot analysis and dramatically reduce PFU/genome ratio. SR has generated analogs and prodrugs of these compounds in an effort to improve efficacy and pK properties for *in vivo* experimentation. We are hoping to perform pK and efficacy testing in 2020 for one or two nucleoside analogs.

Training Plan

(b) (6), (b) (3) (A) (Post-Doctoral Fellow- (b) (6), (b) (3) (A)

We have designed an individual training plan for (b) (6), (b) (3) (A) that will promote (b) (6), career development by directly addressing strengths and weaknesses in (b) (6), (b) (3) scientific and academic aptitudes. As a postdoctoral fellow (b) (6), (b) (3) will have access to the OHSU office for postdoctoral affairs (http://www.ohsu.edu/xd/research/postdocs-students/postdoctoral-fellows-guide/index.cfm). The mission of OHSU's Office of Postdoctoral Affairs (OPA) is to support the career development of all OHSU postdoctoral scholars from arrival to departure. In doing so, the OPA strengthens the research training for all postdoctoral scholars, ensures a consistent and superior postdoctoral experience, and prepares postdoctoral scholars for any professional endeavor they wish to pursue. The most important goal for mentoring students and postdoctoral fellows is to provide them with the skills and experience necessary to be successful at the next level by meeting the following objectives: a) Possess a broad base of established and evolving discipline- and research-specific knowledge and skills; b) Demonstrate effective professional and interpersonal communication skills that allow for interaction with colleagues at all levels; c) Adhere to and reflect professionalism standards and practices within the workplace, institution, and discipline; d) Possess the skills and techniques needed to facilitate effective mentorship, leadership, and management of projects and people; e) Receive training in the responsible conduct of research to improve the ability to make ethical and legal choices; and f) Articulate a plan to pursue a career path of his or (b) (6), (b) (3) choosing.

(b) (6), (b) (3) (A) presents (b) (6), (b) (3) lab work at our formal weekly VGTI meetings, MMI seminars as well as at our combined informal 'chalk talk' style lab meetings. (b) (6), (b) (3) (A) also attends and presents during our Virology/Immunology Journal Club that meets weekly throughout the year. In addition, we have weekly seminars from outside experts, and post-docs are given opportunities to meet the speakers and discuss their work. Last, (b) (6), (b) (3) (A) is expected to present (b) (6), research findings at National and International meetings and attend career development meetings provided by OHSU. The VGTI is an excellent training environment for postdoctoral fellows. The opportunities for learning are immense and the combined experience of the faculty is incredible. The VGTI is an amazing environment that provides a great scaffold for learning how to do 'Team Science'.

(b) (6), (b) (3) (A) (Post-Doctoral Fellow- (b) (6), (b) (3) (A)

An individual training plan is being implemented for (b) (6), (b) (3) (A) that promotes (b) (6), (b) (3) career development by directly addressing strengths and weaknesses in (b) (6), scientific and academic skill sets. All OHSU postdoctoral fellows have access to the OHSU office for postdoctoral affairs (OPA) (http://www.ohsu.edu/xd/research/postdocs-students/postdoctoral-fellows-guide/index.cfm) whose mission is to support career development, research opportunities, and consult on obstacles inherent to postdoctoral training. In these ways OPA strengthens training experience for postdoctoral scholars, facilitates a rewarding postdoctoral experience, and prepares scholars for selecting and pursuing professional endeavors.

Specific goals for postdoctoral training include: 1) Exposure to and refinement of skills needed for experimental inquiry; 2) Support for and emphasis on maintaining a current understanding of rapidly evolving discipline- and topic-specific knowledge; 3) Development of effective interpersonal communication skills that allow for engagement with colleagues at all levels; 4) Understanding adherence to standards of professionalism within the laboratory, institution, and field; 5) Exposure to opportunities that develop effective methods of mentorship, leadership, and project management; 6) Acquire training in the responsible conduct of research to improve make ethical and institutionally compliant choices; and 7) Identification of a career path consistent with the scholar's aspiration.

(b) (6), (b) (3) presents (b) (6), lab work at our formal VGTI meetings once a year as well as at informal work-in-progress style open lab meetings. (b) (6), (b) (3) also attends and presents during a Virology/Immunology Journal Club that meets weekly to stay current in the literature. In addition, VGTI hosts regular seminars from external experts and post-docs are provided opportunities to meet the speakers informally. Lastly, (b) (6), (b) (3) is expected to present her research findings at National and International meetings and attend career development meetings provided by OHSU. The VGTI is an excellent training environment for postdoctoral fellows. The opportunities for learning are immense and the combined experience of the faculty is outstanding.

(b) (6), (b) (3) (A) (Post-Doctoral Fellow- (b) (6), (b) (3) (A)
(b) (6), (b) (3) (A) has worked with (b) (6), (b) (3) (A) for just over (b) (6), (b) (3) years and we have designed a training program that is specifically tailored to promote (b) (6), (b) (3) (A) career development. (b) (6), (b) (3) (A) has successfully trained seven postdoctoral fellows, all of whom continue to pursue scientific careers in academic, government, or industry related research. Based on this experience, (b) (6), (b) (3) (A) has developed a training program with (b) (6), (b) (3) (A) that is designed to facilitate (b) (6), (b) (3) (A) career development and allow him to meet his long-term goals of becoming an independent investigator. This includes the use of an Individual Development Plan (IDP) designed to facilitate discussion of (b) (6), (b) (3) (A) career goals, while also identifying specific training areas where (b) (6), (b) (3) (A) needs additional development. (b) (6), (b) (3) (A) also has weekly meetings with (b) (6), (b) (3) (A) to discuss scientific progress/plans on his research project, career development, and research ethics. (b) (6), (b) (3) (A) also participates in and presents at a weekly (b) (6), (b) (3) (A) meeting, a weekly Virology in Progress meeting involving 25+ virology labs at UNC, and a weekly Department of Genetics Research Colloquium. He also attends weekly research seminar series sponsored by the Departments of Genetics and Microbiology and Immunology, and attends a yearly Virology Colloquium. (b) (6), (b) (3) (A) also attends and presents his research at one or more international research meetings each year.

In addition to the specific training that Dr. Sarkar receives in the Heise lab, as a postdoctoral fellow at UNC, (b) (6), (b) (3) (A) has full access the UNC Office of Postdoctoral Affairs (OPA) https://research.unc.edu › postdoctoral-affairs.
The UNC OPA provides postdoctoral trainees with a comprehensive set of training and support resources. This includes access to career counseling, workshops on career development and communication, and training in Responsible Conduct of Research (RCR). Importantly, (b) (6), (b) (3) (A) is the Faculty Advisor for the UNC OPA, and directly participates in many of OPA's training activities, providing with (b) (6), (b) (3) (A) with additional opportunities to take advantage of the opportunities and resources provided by OPA.

C. COMPONENT PRODUCTS

C.1 PUBLICATIONS

Not Applicable

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Not Applicable

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Not Applicable

C.5 OTHER PRODUCTS AND RESOURCE SHARING

Category	Explanation
Research Material	Mayaro virus (MAYV) infectious clone: The Heise lab, in collaboration with Dr. Streblow's Lab, have sequenced the MAYV BA strain and constructed an infectious cDNA clone of the virus. This clone, and any derivatives containing viral variants will be distributed to the other labs within the Program and made available to the scientific community upon request.

D. COMPONENT PARTICIPANTS

Not Applicable

E. COMPONENT IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

Not Applicable

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

NOTHING TO REPORT

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?
Not Applicable

F. COMPONENT CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. COMPONENT SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

Not Applicable

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Not Applicable

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT

Not Applicable

G.10 ESTIMATED UNOBLIGATED BALANCE

Not Applicable

G.11 PROGRAM INCOME

Not Applicable

G.12 F&A COSTS

Not Applicable

RESEARCH & RELATED BUDGET - SECTION A & B FINAL

ORGANIZATIONAL DUNS*: 0969975150000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: OREGON HEALTH AND SCIENCE UNIVERSITY

Start Date*: 03-01-2020 **End Date*:** 02-28-2021

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		Daniel		Streblow	PhD	Site Principal Investigator	(b) (6), (b) (4)	(b) (6)			22,455.00	7,859.00	30,314.00
2.		(b) (3) (A), (b) (6)			PhD	Co-Investigator					19,344.00	7,157.00	26,501.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **56,815.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
3	Post Doctoral Associates	(b) (6)			63,348.00	19,159.00	82,507.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
5	1 Assistant Staff Scientist and 4 Researchers				94,893.00	35,660.00	130,553.00
8	**Total Number Other Personnel**					**Total Other Personnel**	**213,060.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**269,875.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E

ORGANIZATIONAL DUNS*: 0969975150000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: OREGON HEALTH AND SCIENCE UNIVERSITY

Start Date*: 03-01-2020 **End Date*:** 02-28-2021

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	2,000.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**2,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		0.00
2. Stipends		0.00
3. Travel		0.00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K

ORGANIZATIONAL DUNS*: 0969975150000
Budget Type*: ● Project ○ Subaward/Consortium
Enter name of Organization: OREGON HEALTH AND SCIENCE UNIVERSITY

Start Date*: 03-01-2020 **End Date*:** 02-28-2021

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	55,324.00
2. Publication Costs	2,000.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8. Other Costs: Mice Per Diem	4,000.00
Total Other Direct Costs	**61,324.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**333,199.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. MTDC	54.0	333,199.00	179,928.00
		Total Indirect Costs	**179,928.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Arif M. Karim, 415-437-7820

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**513,127.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification* File Name: Budget Just Streblow U19AI142759 Proj 2.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

BUDGET JUSTIFICATION YEAR 2 – OREGON HEALTH & SCIENCE UNIVERSITY

PERSONNEL ($269,875 Year 2)

Key Personnel:

Daniel N. Streblow, Ph.D. (Multiple-Principal Investigator/Contact PI, Years 1-5: (b)(6) effort, (b)(6) calendar months), is a Professor in the Vaccine and Gene Therapy Institute (VGTI) at OHSU, with an adjunct position in the Division of Pathobiology and Immunology at the Oregon National Primate Research Center (ONPRC). Dr. Streblow will be the Multiple-PI for Project 3 with (b)(3)(A), (b)(6). Dr. Streblow will provide the oversight and organizational structure to ensure clear lines of authority and oversight of research activities, including those of all key personnel and technical staff. He will have responsibility for planning and maintaining communication with the other Projects and Cores and provide a direct line of communication with SR. Dr. Streblow will be responsible for helping to ensure compliance with all federal and NIH regulations as well as the safety and security of data, materials, facilities and resources of the program. Dr. Streblow has extensive experience with *in vitro* and *in vivo* animal models of alphavirus infection and disease as well as a background in pharmacology. Dr. Streblow will be responsible for data analysis and preparation of reports and publications derived from the project.

(b)(3)(A), (b)(6), Ph.D., Co-Investigator (Years 1-5: (b)(6) effort, (b)(6) calendar months). (b)(3)(A), (b)(6) will provide the oversight and organizational structure to ensure proper coordination and efficient execution of research activities in his lab, including those of key personnel, technical staff, and fee-for-service entities. (b)(3)(A), (b)(6) will hold the primary role in promoting scientific direction and interactions, ensuring research progress, and facilitating communications between research personnel, institutions, and the NIH. (b)(3)(A), (b)(6) will be responsible for maintaining compliance with all federal and NIH regulations as well as the safety and security of data, and managing materials, facilities and resources of the program. He has also conceived of, executed, and analyzed results from multi-factor experiments involving generation of large data sets such as *in vitro* high throughput screens and genome-wise transcriptomic profiling and is able to coordinate and troubleshoot all activities related to such undertakings. (b)(3)(A), (b)(6) regularly employs multiple *in vitro* and animal models of immunity, vaccination, and pathogenesis that can be implemented for the proposed studies.

Non-Key Personnel:

(b)(3)(A), (b)(6), Ph.D., Postdoctoral Scholar (Years 1-5: (b)(6) effort, (b)(6) calendar months). (b)(3)(A), (b)(6) is a postdoctoral scientist in the (b)(3)(A), (b)(6). (b)(3)(A), (b)(6) was trained in viral pathogenesis and immunology, and (b)(3)(A), has a strong background in immunology. (b)(3)(A), (b)(6) has provided initial characterization of the SR HTS hit compounds and their analogs. (b)(3)(A), (b) will devote much of her time to characterizing the lead molecules and will perform experiments to test the compounds in alphavirus infected animals. (b)(3)(A), (b)(6) previous work experience makes her an important addition to the group for completion of the proposed experiments.

(b)(3)(A), (b)(6), B.S., Senior Research Assistant (Years 1-3: (b)(6) effort, (b)(6) calendar months; Years 4-5: (b)(6) effort, (b)(6) calendar months). (b)(3)(A), (b)(6) is a technician in the (b)(3)(A), (b)(6). He will be responsible for producing CHIKV and VEEV titered stocks for infection studies, performing dose titration experiments during medicinal chemistry phase, processing of animal samples for quantification of viral loads and assistance where necessary with other analyses. He will also be involved in producing recombinant alphaviruses containing resistance mutations on a wild type infectious clone backbone.

(b)(3)(A), (b)(6), Ph.D., Postdoctoral Scholar (Years 1-5: (b)(6) effort, (b)(6) calendar months). (b)(3)(A), (b)(6) is a postdoctoral scientist in the (b)(3)(A), (b)(6). He will primarily be responsible for executing and interpreting all secondary validation and *in vitro* phenotypic assays. He will also be involved in facilitating animal experiments in coordination with (b)(3)(A), (b)(6) and be centrally involved in statistical data analysis and experimental design.

(b)(3)(A), (b)(6), Research Associate (Years 1-5: (b)(6) effort, (b)(6) calendar months). (b)(3)(A), (b)(6) is an experienced member of the (b)(3)(A), (b)(6) with expertise in molecular diagnostics for CHIKV and VEEV. (b)(3) (b)(3)(A), (b)(6) will aid in the generation of resistance phenotypes and molecular characterization by sequencing. (b)(3)(A), (b)(6) will also aid (b)(3)(A), (b)(6) in compound protection experiments and studies to understand the molecular mechanisms involved in compound mode of action.

(b)(3)(A), (b)(6), Senior Research Assistant (Years 1-3: (b)(6) effort, (b)(6) calendar months and Years 4-5: (b)(6) effort, (b)(6) calendar months). (b)(3)(A), (b)(6) is a researcher and operational manager in the (b)(3)(A), (b)(6). (b)(3)(A), (b) is

responsible for facilitating numerous aspects of laboratory function, including procuring, maintaining, organizing, and curating supplies and reagents, cell line maintenance, and sample storage. (b) (3) (A), (b) will be crucially involved in cell culture, cellular analysis, and molecular analysis (cytokine, RNA, protein). (b) (3) (A), (b) is also responsible for maintaining databases of biological samples and reagents and maximizing research efficiency.

(b) (3) (A), (b) (6), Ph.D., Postdoctoral Scholar (Years 1-5: (b) (6) effort, (b) (6) calendar months). (b) (3) (A), (b) (6) is a postdoctoral scientist in the (b) (3) (A), (b) (6). (b) (3) (A), (b) possesses expertise in the area of innate immune signaling pathways and viral replication. For this (b) (3) (A), is skilled at employing a diverse array of *in vitro* techniques including transgenic cell line construction, CRISPR/Cas9-mediated genome editing, and transcriptomics. (b) (3) (A), (b) is also highly skilled at confocal fluorescence microscopy and cellular imaging techniques. (b) (3) (A), (b) will primarily be responsible for performing and analyzing all IFA experiments, generating RNA samples for RNAseq as well as devising, directing, and troubleshooting all *in vitro* mechanism of action experiments in coordination with (b) (3) (A), (b) (6) and (b) (3) (A), (b).

(b) (3) (A), (b) (6), Ph.D., Assistant Staff Scientist (Years 1-3: (b) (6) effort, (b) (6) calendar months, Years 4-5: (b) (6) effort, (b) (6) calendar months). (b) (3) (A), (b) (6) is a senior researcher in the (b) (3) (A), (b) (6). He possesses expertise in the areas of transgenic cell line construction using multiple technical platforms including lentivectors and transposons and has developed novel methods for optimal in-house application of these methods. He is also highly skilled at CRISPR/Cas9-mediated genome editing and characterization of innate immune signaling pathways and phenotypes. In coordination with (b) (3) (A), (b) (6) he will primarily be responsible for constructing optimal reporter cell lines and generating master cell banks.

(b) (3) (A), (b) (6), M.S., Research Assistant 2 (Years 1-3, (b) (6) effort, (b) (6) calendar months, Years 4-5, (b) (6) effort, (b) (6) calendar months) (b) (3) (A), (b) (6) is a researcher in the (b) (3) (A), (b) (6). (b) (3) (A), (b) possesses an array of talents including molecular cloning, lentivector construction and utilization, CRISPR/Cas9-driven cell line construction, quantitative transcriptional and protein analysis, mouse husbandry and experimentation, and high throughput assays. Under the direction of (b) (3) (A), (b) (6) will be responsible for employing these skills to provide assistance with animal work, processing biomaterials for analysis, construction of cell lines, and molecular biological experiments.

MATERIALS AND SUPPLIES ($55,324 Year 2)

Tissue Culture Supplies: These will be required for all cell growth and maintenance as well as virus growth and titration and isolation from tissues. This includes cell culture growth media, animal serum, PBS, trypsin, sucrose, sorbitol, disposable sterilizing filters, antibiotics, and syringes. ($20,000 in Year 1; $18,000 in Year 2; $15,000 in Year 3; $10,000 in Year 4; and $6,241 in Year 5)

Molecular Biology Reagents: These will be required for all molecular biological and biochemical experimentation, including qPCR (for viral quantification), immunoblotting (to monitor antibody specificity), immunofluorescence, and viral replication monitoring/evaluation. This includes enzymes (e.g., Taq polymerase) and chemicals (e.g., SDS, TRIS base, agarose, TEMED, Tween, BSA, formalin, ethanol, DMSO), agar, and LB broth. ($14,911 in Year 1; $11,324 in year 2; $9,932 in year 3; $9,299 in year 4; $5,198 in year 5).

Antibodies: Antibodies will be required for flow cytometry to identify changes in host lymphocyte phenotype and activation status. ($2,000/yr in Years 1-5)

LiqN_2 & CO_2: CO_2 will be required for all tissue culture. Liquid nitrogen will be required for cryopreservation of all primary cells and cell lines. ($2,000/yr in Years 1-5)

Plasticware: Disposable plasticware will be required for cell and virus culture, virus titration and virus isolation, and molecular biological work. This includes tissue culture dishes of diverse sizes and layouts, flasks, serological pipettes, disposable pipette tips, microfuge and centrifuge tubes, and disposable screw cap tubes of various sizes for sample storage. ($18,000 in year 1; $16,000 in year 2; $12,577 in year 3; $12,468 in year 4; $8,674 in year 5)

Mouse Sample Collection/Drug Administration Supplies: Vacutainer blood tubes, needles, syringes and sterile plastic collection tubes and swabs will be required for obtaining blood samples and tissues. ($1,000/yr in Years 1-5)

Mouse purchase fees: For the testing of compounds in our mouse models we will require up to 250 mice/yr x $20 each = $5,000. We are requesting $5,000 in each year of the project (Years 1-5). Total = $25,000

OTHER EXPENSES ($4,000 Year 2)

Mouse per diems: Per diem costs are $1.20/day x 50 cages (5 animals per cage) x 67 days = $4,000 per year. We are requesting $4,000 in per diem fees for each year of the project. Total = $20,000

PUBLICATIONS ($2,000 Year 2)

Results will be published in peer-reviewed journals as they are satisfactorily completed. ($2,000/yr in Years 1-5)

TRAVEL ($2,000 Year 2)

Travel costs have been allocated for one scientific meeting per year for Drs. Streblow, and (b) (3) (A), (b) (6). This will be necessary to communicate results obtained from the proposed studies and to stay current on related outside research activities and protocols. Funds in the amount of $2,000 annually for Years 1-5 are requested for round-trip airfare, per diems (meals and lodging), meeting registration and miscellaneous travel expenses (parking, ground transportation) based on up to four nights of lodging and five days of per diem. The daily maximum amount for meals and lodging may vary, based on location in high-cost areas.

A. COMPONENT COVER PAGE

Project Title: Project 3.1: Develop Antiviral Agents for Flavivirus Therapy

Component Project Lead Information:

SHI, PEI-YONG

B. COMPONENT ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

SPECIFIC AIMS. The flavivirus genus comprises over 70 positive, single-stranded RNA viruses. Many flaviviruses are significant human pathogens, among which dengue virus (DENV) is the most important mosquito-transmitted virus with over 390 million infections per year. West Nile virus (WNV), yellow fever virus (YFV), Japanese encephalitis virus (JEV), tick-borne encephalitis virus (TBEV), and recently Zika virus (ZIKV) also cause global outbreaks and epidemics, posing constant threats to public health1. No FDA-approved antiviral drug is available to treat any flavivirus infection, and vaccines are lacking against several family members. Thus, the development of flavivirus antiviral therapy is a public health priority.

The goal of this proposal is to develop direct antiviral agents (DAAs) for flavivirus therapy. Antiviral development for all RNA viruses, including flaviviruses, has two major challenges. The first challenge is the rapid emergence of resistance due to the low fidelity of the viral RNA-dependent RNA polymerase (RdRp). Thus, DAAs against different viral targets should be pursued for combination therapy to mitigate resistance, as exemplified by the optimal therapy of hepatitis C virus. During the past funding period of this CETR consortium (AD3C), we identified potent inhibitors of distinct flavivirus protein functions. In the current proposal, we will develop these inhibitors further into clinical candidates through hit-to-lead optimization. These preclinical candidates will form the foundation of combination therapy. The second challenge is the narrow spectrum of antiviral activity due to the sequence variations of the binding pockets of DAAs. Nucleoside/nucleotide (Nuc) DAAs can overcome this challenge through targeting the most conserved active sites of viral polymerases. This strategy has been used successfully to combat human viral infections, as Nuc drugs possess broad-spectrum activity and a high resistance barrier when treating HIV and HCV patients2. We propose to perform a focused testing of novel Nuc collections from the Emory Institute for Drug Development (EIDD; headed by (b) (3) (A), (b) (6)) and (b) (3) (A), (b) (6) against flaviviruses. In support of this idea, testing of the EIDD Nuc collection against coronaviruses and alphaviruses identified promising inhibitors for Projects 1 and 2 of AD3C. Based on the above rationale, we propose three Specific Aims to develop flavivirus DAAs (see Fig 1).

Aim 1. Develop preclinical DAAs targeting flavivirus capsid and NS4B protein. Previous studies have identified promising inhibitors of DENV capsid (required for flavivirus assembly) and ZIKV NS4B (required for flavivirus replication and immune evasion) proteins. We will perform hit-to-lead optimization to advance these compounds to animal efficacy testing and preclinical development. Specifically, (i) the DENV capsid inhibitor showed in vivo efficacy, but low aqueous solubility. We have crystalized DENV-2 capsid protein in complex with the inhibitor, and will perform structure-based rational design to improve its solubility and efficacy. (ii) The ZIKV NS4B compound exhibited <10 nM EC50 and >25 μM CC50 in cell culture. We will profile the pharmacokinetics (PK) and test the in vivo efficacy of the inhibitor in a ZIKV mouse model. For both compound classes, medicinal chemistry will be performed to broaden the antiviral spectrum to other flaviviruses. In addition, we will passage viruses in the presence of drug candidates to define the potential for resistance, followed by characterization of the resistant viruses for their replication fitness in vitro and pathogenic potential in vivo.

Aim 2. Identify novel Nuc DAAs with pan-flavivirus activity. Using DENV as a pilot, we will identify panflavivirus Nuc inhibitors from the EIDD and (b) (3) (A), (b) (6) compound collections. We will test concurrently (i) a nucleoside (that require host kinases to convert to nucleoside triphosphate for antiviral activity) collection in a cell-based DENV infection assay and (ii) a nucleoside triphosphate collection in a biochemical RdRp assay. Compounds with activities in both assays will be prioritized for development because they are efficiently converted to nucleoside triphosphate by host kinases. Compounds without activity in (i), but with activity in (ii) are not efficiently converted to nucleoside triphosphate by host kinases; we will pursue phosphonate or phosphoramidate prodrugs to rescue these Nucs for development. For identified Nucs, we will characterize their spectrum against other flaviviruses, confirm mechanism of action, assess in vivo protection, and evaluate resistance.

Aim 3. Evaluate combination therapy directed against flavivirus infection. Combination therapy is essential to prevent resistance during antiviral treatment. We will test therapeutic regimens combining DAAs with distinct viral targets from Aims 1 and 2. We will first examine the synergistic or additive effects of DAAs in cell culture. The most effective combinations from cell culture will be evaluated in mouse pathogenesis models. Finally, the most promising DAA combination will be validated in non-human primate (NHP) models of flavivirus infection and will be used to guide future clinical implementation.

We will deliver the proposed milestones by combining the cutting-edge biology from academia and drug discovery acumen from industry along with defined Go/No-Go decision algorithms and with input from the Scientific Advisory Committee (SAC). The collective effort addresses a major unmet medical need by bridging academia and industry, including investigators from University of Texas Medical Branch (Pei-Yong Shi), Washington University in St. Louis (b) (3) (A), (b) (6) Oregon Health Science University (b) (3) (A), (b) (6), EIDD (b) (3) (A), (b) (6) Southern Research (Ashish Pathak), and (b) (3) (A), (b) (6)

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: B2 Yr 1 Project 3 flavi annual report Dec 10 2019-sm.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

Not Applicable

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

File uploaded: B4 Yr 1 Project 3 flavi annual report Dec 10 2019-sm.pdf

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

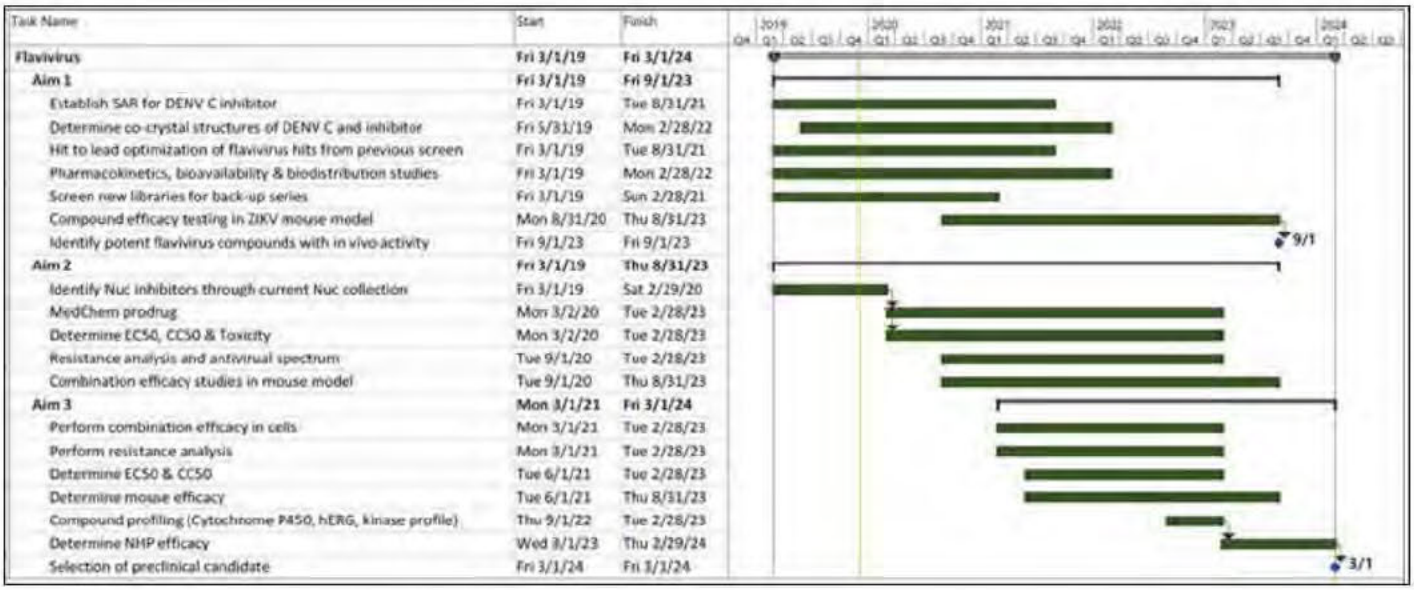
B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

We will pursue the project as proposed in the original grant in the coming year (see the tasks and timeline table in B.2). Briefly, we will continue the following promising directions:
•Perform cocrystal structure-based rational design of dengue capsid inhibitors to achieve improved antiviral spectrum and physicochemical properties.
•Complete hit-to-lead medchem effort on SRI-39748 and SRI-39757 scaffolds (derived from previous screens) to reach go/no-go decision.
•Continue to pursue the (b) (3) (A), (b) (6 compound for in vivo efficacy in Zika mouse model.
•Test in vivo efficacy for three potential pan-flavivirus nucleoside/nucleotide inhibitors (SRI-43918, EIDD-1931/EIDD-2801, and Remdesivir)
•Complete dengue screen of 200,000 compounds

Project 3 B.2 Accomplished under goals:

Major activities and objectives: We have made significant progress on the flavivirus antiviral project. Inhibitors of Zika virus (ZIKV), dengue virus (DENV), and pan-flavivirus are being pursued in parallel. The new chemical entities are derived from Southern Research, [redacted] (b) (6), (b) (3) (A). The proposed project milestones have been met according to the original timelines.

Task Name	Start	Finish
Flavivirus	**Fri 3/1/19**	**Fri 3/1/24**
Aim 1	**Fri 3/1/19**	**Fri 9/1/23**
Establish SAR for DENV C inhibitor	Fri 3/1/19	Tue 8/31/21
Determine co-crystal structures of DENV C and inhibitor	Fri 5/31/19	Mon 2/28/22
Hit to lead optimization of flavivirus hits from previous screen	Fri 3/1/19	Tue 8/31/21
Pharmacokinetics, bioavailability & biodistribution studies	Fri 3/1/19	Mon 2/28/22
Screen new libraries for back-up series	Fri 3/1/19	Sun 2/28/21
Compound efficacy testing in ZIKV mouse model	Mon 8/31/20	Thu 8/31/23
Identify potent flavivirus compounds with in vivo activity	Fri 9/1/23	Fri 9/1/23
Aim 2	**Fri 3/1/19**	**Thu 8/31/23**
Identify Nuc inhibitors through current Nuc collection	Fri 3/1/19	Sat 2/29/20
MedChem prodrug	Mon 3/2/20	Tue 2/28/23
Determine EC50, CC50 & Toxicity	Mon 3/2/20	Tue 2/28/23
Resistance analysis and antiviral spectrum	Tue 9/1/20	Tue 2/28/23
Combination efficacy studies in mouse model	Tue 9/1/20	Thu 8/31/23
Aim 3	**Mon 3/1/21**	**Fri 3/1/24**
Perform combination efficacy in cells	Mon 3/1/21	Tue 2/28/23
Perform resistance analysis	Mon 3/1/21	Tue 2/28/23
Determine EC50 & CC50	Tue 6/1/21	Tue 2/28/23
Determine mouse efficacy	Tue 6/1/21	Thu 8/31/23
Compound profiling (Cytochrome P450, hERG, kinase profile)	Thu 9/1/22	Tue 2/28/23
Determine NHP efficacy	Wed 3/1/23	Thu 2/29/24
Selection of preclinical candidate	Fri 3/1/24	Fri 3/1/24



B.2.a DENV capsid inhibitor: We have solved the cocrystal structure of dengue virus serotype-2 capsid (DENV-2 C) in complex of inhibitor ST148 (1.5 Å resolution; **Fig. 1**). The cocrystal structure has revealed a novel antiviral mechanism to target viral capsid. The structural information has also uncovered the mechanism of resistance and antiviral spectrum. The cocrystal enables rational design of analogs with improved compound solubility and antiviral spectrum.

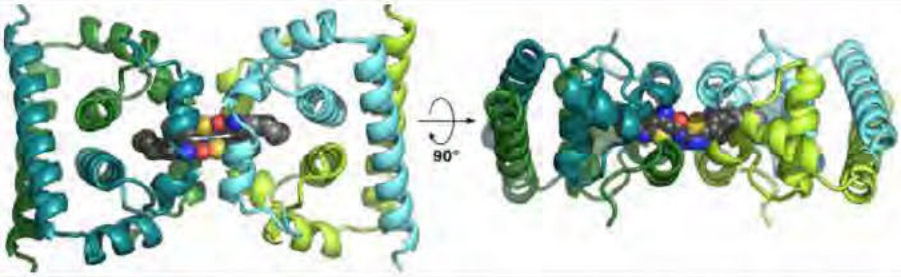
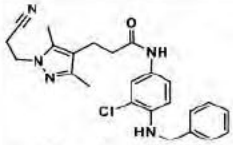
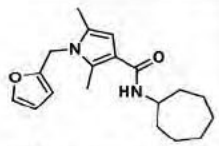


Fig 1. Cocrystal structure of DENV-2 C protein in complex with inhibitor ST148. Structure of DENV-2 C tetramer in complex with ST148. Two C dimers form a "kissing" tetramer. Two ST148 molecules bind to a pocket at the tetramer interface. ST148 and C protein tetramer are presented as CPK and cartoon, respectively.

B.2.b ZIKV inhibitor: Inhibitors identified from previous high-throughput screening have undergone "hit-to-lead" optimizations. Specifically, we have identified two promising scaffolds of ZIKV inhibitors (SRI-39748 & SRI-39757) currently under hit-to-lead optimization. SAR has been established for these scaffolds to minimize the liabilities of these inhibitors.



SRI-39748
EC_{90} 0.13 µM
CC_{50} >40 µM
Solubility 7 µM
LogD 3.2
HLM $t_{1/2}$ 6 min
MLM $t_{1/2}$ 1 min



SRI-39757
EC_{90} 4.7 µM
CC_{50} >40 µM
Solubility 23 µM
LogD 3.7
HLM $t_{1/2}$ 9.5 min
MLM $t_{1/2}$ 2.9 min

We have demonstrated in vivo efficacy for one class of ZIKV inhibitor in A129 mouse model (**Fig. 2**). One inhibitor [redacted] (b) (3) (A), (b) (6) has shown promising mouse efficacy with ~100-fold viremia reduction. In addition, the compound completely prevented weight loss or death of infected mice.

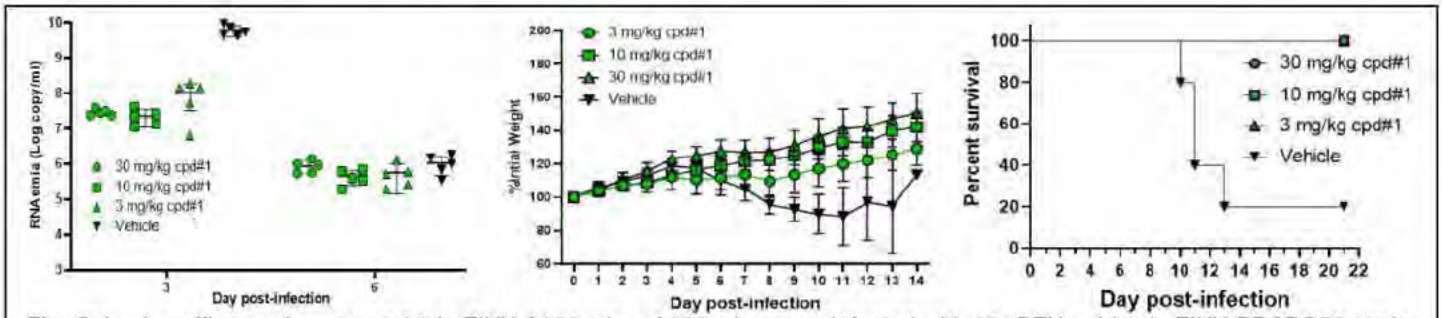
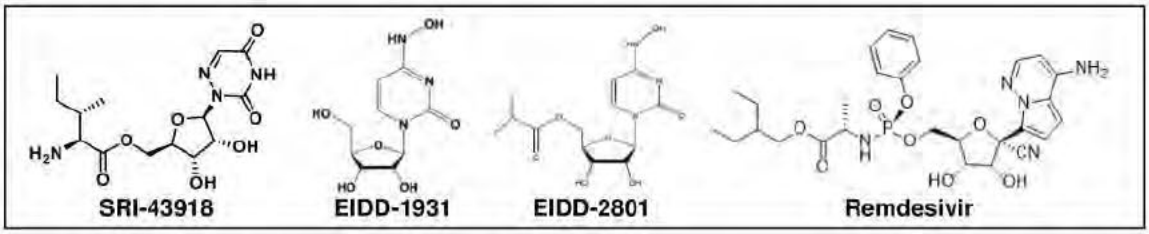


Fig. 2. In vivo efficacy of compound 1 in ZIKV A129 mice. A129 mice were infected with 10_5 PFU epidemic ZIKV PRABC59 strain, immdiately treated daily with indicated dose of compound, and monitered for viremia (left), weight loss (middle), and survival (right).

B.2.c Pan-flavivirus inhibitor: We have taken the following three approaches to identify pan-flavivirus inhibitors. As expected, nucleoside/nucleotide analogs are likely to have pan-flavivirus activities.

- We have identified Southern Research nucleotide inhibitor SRI-43918. SRI-43918 has pan-DENV partitivity with EC_{50} of 1-6 μM and CC50 >50 μM. We are currently verifying the mode-of-action of the compound and planning in vivo efficacy test.



- EIDD-1931 and its ester prodrug EIDD-2801, nucleoside/nucleotide from Emory Drug Innovation Ventures, have been identified with anti-flavivirus activities in cell culture. Optimal dose and route will be explored for in vivo activity.
- We have obtained Remdesivir from Gilead and tested its activity against flaviviruses. We found Remdesivir has pan-flavivirus activity in cell culture against DENV-1 to -4, WNV, JEV, ZIKV, and YFV (EC_{50} 0.1-1.0 μM). The cell culture efficacy justifies to test Remdesivir in WNV and ZIKV mouse models.

It should be noted that ester prodrugs are commonly used to improve nucleoside/nucleotide drug absorption and cellular membrane penetration. In humans, intracellular carboxylesterase degrades the ester moiety and releases the nucleoside/nucleotide for triphosphorylation. Because mice express a high level of secreted carboxylesterase 1c (*Ces1c*, which is absent in human plasma), the secreted carboxylesterase 1c in mouse plasma rapidly degrades the ester moiety of the prodrug before the compound enters cells. Therefore, for compounds with an ester prodrug moiety (such as SRI-43918, EIDD-2801, and Remdesivir), we may have to use *Ces1c*$^{-/-}$ mice for efficacy testing. To overcome this challenge, **we have obtained *Ces1c*$^{-/-}$ mice from** (b) (3) (A), (b) (6) Besides direct use for efficacy testing, the *Ces1c*$^{-/-}$ mice will be crossed with A129 or AG129 mice to create double knockout mice for DENV and/or ZIKV testing. The double knockout mice will be a critical reagent for the field of antiviral research.

B.2.d Screening effort for new dengue inhibitors. To ensure a robust flavivirus pipeline, we have initiated a new screen of 200,000 compounds. These compounds have not been tested in our previous screen. We will prioritize compounds with pan-DENV or pan-flavivirus activities. In addition, due to the sequence variations among the four serotypes of DENV, we plan to perform a pilot screen using DENV-4. This is because DENV-4 is more phylogenetically distant from DENV-2 that has been the only serotype for dengue drug discovery in the past two decades. We believe a DENV-4 screen may identify hits with different antiviral profiles.

Project 3 B.4 Training opportunities: All students, postdocs, and technicians have had the opportunity to learn real drug discovery through this project. These hands-on experiences are invaluable for their career development and future research selection. Besides performing experiments, they also participate in data analysis, project discussion, manuscript preparation, and presentation at conferences. These trainees are well positioned for potential industry career opportunities.

C. COMPONENT PRODUCTS

C.1 PUBLICATIONS

Not Applicable

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Not Applicable

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Not Applicable

C.5 OTHER PRODUCTS AND RESOURCE SHARING

Nothing to report

D. COMPONENT PARTICIPANTS

Not Applicable

E. COMPONENT IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

Not Applicable

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

NOTHING TO REPORT

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?
Not Applicable

F. COMPONENT CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. COMPONENT SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

Not Applicable

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Not Applicable

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT

Not Applicable

G.10 ESTIMATED UNOBLIGATED BALANCE

Not Applicable

G.11 PROGRAM INCOME

Not Applicable

G.12 F&A COSTS

Not Applicable

RESEARCH & RELATED BUDGET - SECTION A & B FINAL

ORGANIZATIONAL DUNS*: 8007711490000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: University of Texas Medical Branch at Galveston

Start Date*: 03-01-2020 **End Date*:** 02-28-2021

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Pei Yong		Shi		Project Lead	(b) (6), (b) (4)	(b) (6)			28,845.00	4,304.00	33,149.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **33,149.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
2	Post Doctoral Associates	(b) (6)			102,900.00	37,249.00	140,149.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
2	**Total Number Other Personnel**					**Total Other Personnel**	**140,149.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**173,298.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E

ORGANIZATIONAL DUNS*: 8007711490000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: University of Texas Medical Branch at Galveston

Start Date*: 03-01-2020 **End Date*:** 02-28-2021

C. Equipment Description		
List items and dollar amount for each item exceeding $5,000		
Equipment Item		**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file		**0.00**
	Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	1,500.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**1,500.00**

E. Participant/Trainee Support Costs		Funds Requested ($)*
1. Tuition/Fees/Health Insurance		0.00
2. Stipends		0.00
3. Travel		0.00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K

ORGANIZATIONAL DUNS*: 8007711490000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: University of Texas Medical Branch at Galveston

Start Date*: 03-01-2020 **End Date*:** 02-28-2021

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	25,345.00
2. Publication Costs	3,000.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
Total Other Direct Costs	**28,345.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**203,143.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. MTDC	58.0	203,143.00	117,823.00
		Total Indirect Costs	**117,823.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Arif Karim; Phone: 214-767-3261

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**320,966.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification* File Name: Budget Justification UTMB Dec 16 2019.pdf

(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

UTMB Year 2 Budget justification

Personnel:

Pei-Yong Shi, PhD, PI, will devote (b) (6) calendar months effort and receive (b) (6) calendar month's salary support. Dr. Shi is a recognized leader in the field of antiviral drug discovery and flavivirus biology. He will oversee the flavivirus project and work closely with (b) (3) (A), (b) (6), and other team members to deliver the proposed milestones. His team at UTMB will perform DENV capsid crystal structure in complex with inhibitor, ZIKV inhibitor analysis, and Nuc characterization. In addition, together with other key members of the project, he will be responsible for generating progress reports and manuscripts.

(b) (3) (A), (b) (6) Ph.D. Post-doctoral fellow, will devote (b) (6) calendar months and receive (b) (6) calendar month's salary support (b) (3) (A), (b) (6) has a strong track record in flavivirus biology and drug discovery (b) (3) (A) identified dengue viral NS4B dimerization, NS3/NS4B interaction, and NS4A/NS4B interaction. (b) (3) (A), (b) (6) will perform all experiments on compound testing and mode-of-action study. (b) (3) (A), (b) (6) has expertise in biochemistry, virology, and animal model.

(b) (3) (A), (b) (6), Ph.D. Post-doctoral fellow, will devote (b) (6) calendar months and receive (b) (6) calendar month's salary support. (b) (3) (A) (b) (6) will be responsible for all experiments related to DENV capsid inhibitors. He has solved the co-crystal structure of DENV-2 capsid protein in complex with ST-148 inhibitors. (b) (3) (A) (b) (6) has expertise in both biochemistry and molecular virology. He will test compound efficacy, cytotoxicity, and resistance analysis.

Travel: $1,500
The travel funds are needed to attend the annual meeting of the project.

Reagents: $25,345
PCR reagents, PCR purification kits, plasmid purification kits, cell culture medium, cell culture flasks, electroporation reagents, antibodies, restriction enzymes for DNA cloning, DNA sequencing

Publication fee: $3,000

A. COMPONENT COVER PAGE

Project Title: Project 4.1: Identify New Therapies Targeting Influenza Replication

Component Project Lead Information:

WHITLEY, RICHARD J.

B. COMPONENT ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

SPECIFIC AIMS
Specific aims: The overall goal of this project is to identify new therapies that target influenza virus replication. The global health burden of annual influenza infections and periodic epidemics coupled with the emergence of avian influenza viruses (AIVs), like H5NX and H7N9, highlight the urgent need for new effective treatments. A primary concern with the current influenza therapies (M2 and neuraminidase inhibitors [NAIs]) is the development of resistance mutations that can negate therapeutic benefit. Both published evidence and clinical experience suggest strongly that targeting the influenza virus RNA dependent RNA polymerase (RdRp) complex is a rational approach for antiviral therapy. This complex is responsible for many viral functions, including 5´ cap recognition, endonucleolytic cleavage, RNA synthesis, and polyadenylation. Clearly, the trimeric RdRp complex provides multiple functional domains as targets for antiviral drug therapy and combination therapy. Agents can target the distinct functions and, theoretically, reduce the minimize development of resistance since resistance mutations would likely reduce the fidelity of the RdRp. One oral agent targeting the endonuclease domain, baloxavir, has shown efficacy in uncomplicated influenza and was recently approved in Japan for treating influenza in adults and children. A second oral agent targeting the 5´ cap binding domain, pimodivir, has shown antiviral activity in uncomplicated influenza and is advancing in clinical development. A third oral agent, favipiravir, targeting the polymerase activity has been approved in Japan for treating novel influenza strains not inhibited by NAIs.

We have recently identified several potent molecules from the Emory Institute for Drug Discovery (EIDD), (b) (3) (A), (b) (6) and Southern Research (SR). This application and research team provides the medicinal chemistry expertise, follow up assays, and In vivo experience to transform active hits into lead compounds and advance them rapidly into animal models of influenza virus infection. The public private partnership with EIDD and GS leverages the proposed developmental program and promises to yield new classes of highly active
molecules that target the RdRp complex. We propose the following Specific Aims:

Aim 1. Identify potent influenza compounds targeting RdRp complex functions
A. Continue hit to lead optimization of the active compounds from EIDD, GS, and SR.

Hypothesis and rationale: We hypothesize that hits identified from ongoing studies that target the RdRp complex can serve as the basis for a lead optimization program. This includes novel chemical classes from EIDD, SR, and GS. We will use an iterative medicinal chemistry effort that addresses potency (antiviral activity) as part of the Medicinal Chemistry and Lead Development Core (MCLDC). The focus will be on the essential activities of this complex, which provides fertile ground for drug discovery due to the number of conserved functional domains, and interactions that could be targeted. Our goal is to identify compounds directed against the conserved functions of the RdRp that are active against multiple subtypes of influenza virus.

Approach: The initial hits from EIDD, GS, and the SR compound collection will undergo hit to lead chemistry. Additional analogs will be evaluated and related compounds synthesized. Compounds with effective concentrations below 5 µM will be evaluated further to assess their activity against multiple subtypes of the virus.

Aim 2: Define the spectrum of antiviral activity and define mechanism of action of the lead compounds
A. Define spectrum of antiviral activity against wild-type, AIV, and NAI resistant strains of influenza virus
B. Characterize the mechanism of action (MOA) against RdRp functions
C. Map molecular determinants of resistance and their impact on susceptibility and replication fitness
D. Perform combination studies and evaluate lead molecules from other three Projects

Hypothesis and rationale: Active molecules presented here will be evaluated to confirm their activity against multiple subtypes of the virus including AIVs and preliminary data already have identified compounds that appear to inhibit at least three distinct RdRp functions. Additional assays in an appropriate setting of primary human small airway epithelial cells and biochemical assays will identify domains targeted by the most active compounds.

Experimental strategy: Several secondary and tertiary assays are in place to identify rapidly compounds that specifically inhibit the functions of the RdRp complex. The lead compounds also will be used to generate resistant isolates so that the molecular determinants of resistance can be mapped and confirmed in engineered viruses.

Aim 3: In vivo evaluation and bioavailability studies.
A. Perform maximum tolerated dose and pharmacokinetic studies in mice
B. Evaluate efficacy against mouse-adapted A/California/04/2009 (H1N1) infection in mice
C. Characterize efficacy of lead compound in ferrets infected with A/California/04/2009 (H1N1)

Hypothesis and rationale: Pharmacokinetic, bioavailability, distribution, and clearance studies will prioritize the best chemical scaffolds for further development and help inform the design of studies in mice and ferrets for pivotal efficacy experiments In vivo.

Experimental strategy: Compounds with the best efficacy from Aims 1 and 2 will be prioritized to identify molecules with drug-like properties for efficacy studies in murine and ferret models of influenza virus infection.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: B.2 AD3C Project 4-Y1report.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

Not Applicable

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

NOTHING TO REPORT

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

•Initiate hit-to-lead studies on 1-2 HTS hits.
•Synthesis and evaluation of additional Pimodivir analogs in RdRp, CPE and VLR assays.
•Continue SAR on nucleoside SRI-7958 to improve anti-flu activity.
•Screen compounds (all projects) for DHODH inhibition.
•Establish RdRp cell free assay.
•New RdRp target: ANP32A-PB2 interactions for anti-flu drug discovery
•Perform NMR docking to map active sites
•Characterize mechanism of action of lead compounds: RpRd functions
•Combination Studies: Efficacy of best lead compounds will be evaluated in combination with oseltamivir as well as with baloxavir, pimodivir, and favipiravir.
•In vivo evaluation of 1-2 lead compounds in mouse models of influenza

B.2 Project 4: Identification and Characterization of Novel Drugs that Target the Influenza Virus Polymerase Functions

General Progress summary

- HTS Screening of 200,000 compounds (ChemDiv collection) using a Mirrorball immunofluorescent assay to measure M2, identified 140 confirmed hits with IC_{50} < 30μM and CC_{50} > 40μM.
- 31 compounds from HTS screening were selected for follow-up evaluation after clustering analysis, pain filtration and visual inspections of the structures of the compounds.
- 28 compounds were procured; 26 compounds passed QC tests and screened against H3N2 and H1N1.
- 11 compounds were active against H3N2 and only one compound was also active against H1N1. None of the 11 compounds from HTS were active against Dihydroorotate Dehydrogenase (DHODH).
- SRI-44249 (active against both H1N1 & H3N2) selected for re-synthesis and follow-up
- SRI-44218, SRI-44251, SRI-44209, SRI-44213 also selected for potential follow-up studies
- 18 Nucleoside analogs screened against H3N2; 5 analogs active, no DHODH inhibition.
- DHODH counter screen assay established with recombinant hDHODH enzyme.
- Recombinant human DHODH was produced at Southern Research.
- Mini genome and Cap snatching RdRp assays were standardized.

Antiviral Assays

- Mirrorball® immunofluorescence assay detecting surface expression of M2 protein (H3N2 & H1N1/A549) (HTS and SAR) (Southern Research)
- CPE Assay (H3N2 & H1N1/MDCK) (SAR and Follow-up) (Southern Research)
- Virus Load Reduction assay (H1N1/A549-MDCK) (Follow-up) (UAB)

Mechanism of Action/Molecular Target Assays

- RdRp Cap snatching assay (UAB)
- Mini-genome cell based transcription assay (Southern Research)
- Human Dihydroorotate Dehydrogenase (DHODH) inhibition assay was setup as a counter screen to exclude the DHODH inhibitors from further follow-up. (Southern Research)

SAR Driving Assays

- 131 novel compounds tested (March-October, 2019) at ten concentrations
- Mirrorball IFA used to determine IC_{50} values against H3N2 (Udorn) and H1N1 (Wisconsin)
- Evaluation of cytotoxic effect on A549 cells at 72 hours; used to determine CC_{50} values

HTS Campaign

- Paucity of hits from previous screens using the ELVIRA luciferase reporter assay and a CPE assay (screened Enamine and ChemBridge compound libraries)

- Additional chemical matter needed for the Influenza project
- Screened another 200,000 compounds (ChemDiv collection) using a Mirrorball immunofluorescent assay to measure M2 protein levels in H3N2 infected A549 cells (same assay used to drive SAR)
- Identified 140 confirmed hits with IC_{50} < 30μM and CC_{50} > 40μM
- 10 VX787 analogs tested in RdRp assays and also against H3N2; one analog (SRI-43679) active. SRI-43679 was also active against H1N1 and H3N2. None of the 10 VX787 analogs were active against DHODH.
- There were 125 compounds tested against DHODH counter-screen; 23 active compounds were tested for dose-response to determine relative selectivity (antiviral activity vs DHODH inhibition).
- EIDD-2838 is a broadly active ribonucleotide-5'-monophosphate analog with activity against influenza A and B strains (EC_{50} ~1μM) in CPE models of infection. EIDD-2838 was found to be an influenza cap-snatching inhibitor (EC_{50} = 2-4 μM) through the AD3C.

C. COMPONENT PRODUCTS

C.1 PUBLICATIONS

Not Applicable

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Not Applicable

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Not Applicable

C.5 OTHER PRODUCTS AND RESOURCE SHARING

Nothing to report

D. COMPONENT PARTICIPANTS

Not Applicable

E. COMPONENT IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

Not Applicable

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

NOTHING TO REPORT

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?
Not Applicable

F. COMPONENT CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. COMPONENT SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

Not Applicable

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Not Applicable

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT

Not Applicable

G.10 ESTIMATED UNOBLIGATED BALANCE

Not Applicable

G.11 PROGRAM INCOME

Not Applicable

G.12 F&A COSTS

Not Applicable

RESEARCH & RELATED BUDGET - SECTION A & B FINAL

ORGANIZATIONAL DUNS*: 063690705

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2020 **End Date*:** 02-28-2021

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		Richard		Whitley		Project Lead	(b) (6), (b) (4)	(b) (6)			34,614.00	9,761.00	44,375.00
2.		(b) (3) (A), (b) (6)				Co-investigator					11,783.00	3,323.00	15,106.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **59,481.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
2	1 Research Supervsr/1 Research Tech	(b) (6)			34,929.00	11,457.00	46,386.00
2	**Total Number Other Personnel**					**Total Other Personnel**	**46,386.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**105,867.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E

ORGANIZATIONAL DUNS*: 063690705

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2020 **End Date*:** 02-28-2021

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	1,500.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**1,500.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		0.00
2. Stipends		0.00
3. Travel		0.00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K

ORGANIZATIONAL DUNS*: 063690705

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2020 **End Date*:** 02-28-2021

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	23,909.00
2. Publication Costs	1,500.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8. Other direct - repair & maintenance	1,250.00
9. Sequencing	5,250.00
Total Other Direct Costs	**31,909.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**139,276.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. MTDC	48.5	139,276.00	67,549.00
		Total Indirect Costs	**67,549.00**

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**206,825.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification*	File Name: Whitley Project 4.1 Budget justification Year 2_mw.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Program Director/Principal Investigator (Last, First, Middle): Whitley, Richard J. (Project 4.1)

Budget Justification

Personnel

Richard J. Whitley, MD, PD/PI, (b) (6) calendar months: Dr. Whitley will continue to serve as the UAB Program Director/Principal Investigator for Project 4 investigating the inhibitors of influenza virus. He directs the activities of the research staff, and meets regularly with both Project and Core staff to provide guidance and insure timelines and aims are being met. He continues to provide broad oversight of the project, and serves as primary liaison with the External Advisory Board, NIH and other external entities, including pharmaceutical companies to determine potential compounds to be developed, and international groups such as the IDSA to determine therapeutic needs.

(b) (3) (A), (b) (6), **PhD. (b) (6) calendar months**: (b) (3) (A), (b) (6) has more than (b) (3) years of experience in molecular virology and structural biology focusing on influenza, and, in addition to this project, he currently has an active NIAID funded R01 studying the non-structural protein 1 in influenza for docking studies and antiviral compounds. He will serve as a co-investigator for Project 4 and will assist with development of structural and *in silico* methods that can be used to determine the mechanism of action for lead compounds to inform structure-based modification of compounds against influenza RNA-dependent RNA-polymerase. The scientific advisory committee has suggested the use of structure based studies in all projects, and (b) (3) (A), (b) (6) expertise will provide this function to Project 4.

(b) (3) (A), (b) (6), **MS, Research Laboratory Supervisor, (b) (6) calendar months,**: (b) (3) (A), (b) (6) has more than (b) (3) years of experience in laboratory work on a number of viruses including HIV, α&β-herpes, influenza, vaccinia and cowpox primarily determining in vitro drug efficacy using different endpoint methods (e.g., ELISA, CPE, plaque reduction, virus yield, hybridization, real time PCR) and under Biosafety levels 2 - 3. (b) (3) will continue to oversee the antiviral assays and day to day activities for this project in (b) (3) (A), (b) (6).

(b) (3) (A), (b) (6), **Research Technician, (b) (6) calendar months**, (b) (3) (A), (b) (6) has more than (b) (3) years of experience in research labs and has increased activity on this project as work has expanded on the assays and in vitro lab work. . (b) (3) will continue to provide general assistance with day to day operations.

Supplies

Funds ($23,909) are requested for tissue culture, reagents, surgical supplies, PPE, lab ware and miscellaneous laboratory supplies need to conduct the planned compound testing.

Travel

Funds ($1,500) are requested to allow travel of the PD/PI and co-investigators to attend ICAR or other related scientific meetings to present results of the project

Other Expenses

Funds are requested for shipping of materials ($250), publication costs ($1,500), and maintenance of project specific equipment ($1,000). Funding for sequencing core services ($5250) is also included.

Indirect Costs

Indirect costs are calculated on modified total direct costs at the federally approved rate of 48.5%

A. COMPONENT COVER PAGE

Project Title: Project 1.2: The Development of Antivirals for Coronavirus Replication

Component Project Lead Information:

Baric, Ralph S

B. COMPONENT ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

SPECIFIC AIMS
Zoonotic coronaviruses (CoVs) such as severe acute respiratory syndrome (SARS)-CoV and Middle East respiratory syndrome (MERS)-CoV are pandemic threats, with MERS-CoV continuing to cause new zoonotic and human transmission and illness, resulting in ~35% mortality. Currently, there are no FDA-approved therapies to treat any CoV. Several challenges for anti-CoV drug development exist. 1) As with SARS-CoV and MERSCoV, new zoonotic CoVs likely will emerge from divergent virus pools in animal reservoirs, thus requiring antiviral strategies aimed at conserved targets. 2) Although nucleoside analogues/mutagens are effective against many RNA viruses by acting against viral RNA-dependent RNA polymerases, CoVs uniquely encode a novel RNAdependent proofreading exoribonuclease (nsp14-ExoN), thus requiring effective nucleoside analogues to inhibit or resist the action of ExoN. 3) CoVs are able to rapidly develop resistance to multiple classes of inhibitors, demonstrating the need for approaches to prevent resistance emergence. 4) Both SARS and MERS progress to severe immunopathologic damage, potentially limiting the therapeutic window for direct-acting antivirals (DAAs). Thus, combinations of DAAs and targeted immunomodulators may be necessary for effective treatment of established infection.

The overall goal of our program is to develop CoV antiviral strategies that broadly inhibit known and future potential pandemic zoonotic CoVs, prevent emergence of resistance, and extend the therapeutic window by targeting host immunopathologic responses. Our previous work identified nucleoside analogues (NAs) and non-nucleoside compounds with potent activity against CoVs. Two lead NAs in advanced stages of preclinical development, remdesivir (GS-5734, Gilead Sciences) and EIDD-1931/2801 (Emory Institute for Drug Development), inhibit multiple CoVs, including SARS-CoV and MERS-CoV, in the presence of the intact ExoN. Other promising NAs are also in the pipeline. Two hits from screening at Southern Research (SR), SR-36097 and SR-35293, dramatically inhibit SARS-CoV replication in cell monoculture and in primary human airway epithelial cell cultures. The proposed research will advance preclinical development of the CoV-inhibitory EIDD and SR compounds for treatment and prevention of epidemic and pre-emergent CoVs, generating mechanistic and efficacy data necessary for IND filing and origination of human clinical trials. In addition, we will explore additive or synergistic effects of DAAs. Finally, we will establish proof-of-concept testing for combination of DAAs and immunomodulatory drugs to extend the therapeutic window and treat established infection in vivo. The impact of our program is high. Advancing new leads, increasing potency, broadening activity spectrum, expanding the therapeutic window, and preventing resistance of antiviral treatments for epidemic CoVs addresses an immediate unmet medical need for MERS-CoV and could prove to be an invaluable tool to prevent future pandemics due to pre-emergent CoVs and other high-consequence viral pathogens.

Aim 1. To determine the spectrum of antiviral activity and therapeutic efficacy of EIDD-1931/2801 and perform hit-to-lead stage evaluation of SR compounds. In this aim, we will (i) determine EIDD-1931 and
other DAA activity spectra across CoV groups and genera; (ii) determine antiviral efficacy, metabolism, and cytotoxicity in human lung cells targeted by SARS- and MERS-CoV; (iii) evaluate the efficacy of EIDD-1931 and other DAA NA in young, aged, and immunosuppressed murine models of MERS and SARS pathogenesis; and (iv) perform hit-to-lead studies for SR compounds. SR compounds that meet all Go/No-Go criteria will be entered as lead compounds into the development pipeline as described for EIDD-1931/2801.

Aim 2. To define mechanism of action of EIDD-1931/2801 and impact of resistance mutations on in vivo therapeutic efficacy, viral fitness, and pathogenesis. In this aim, we will (i) define lead compound
mechanisms of action by assessing the impact on CoV replication, chain termination, fidelity, and innate immune responses; (ii) assess the kinetics of drug-resistance development and impact of drug resistance on viral replication and fitness; (iii) determine the effect of drug resistance on sensitivity to other drugs; (iv) determine the impact of resistance mutations on MERS-CoV and SARS-CoV replication, fitness, and pathogenicity in mice; and (v) determine the impact of resistance mutations on therapeutic efficacy. Data collected in Aims 1 and 2 are crucial prerequisites to progression of lead compounds to clinical evaluation.

Aim 3. To test combination regimens for treatment of emerging CoV disease. Emerging coronaviruses evolve quickly and can cause acute severe end-stage immunopathologic lung disease (ARDS), which potentially limits therapeutic windows. Thus, new strategies are needed to extend the effective therapeutic window of DAAs. In this aim, we will (i) assess the combined efficacy and potency of DAAs against MERS-CoV and SARS-CoV in vitro; (ii) evaluate therapeutic and prophylactic efficacy of combined DAAs in murine models of MERS and SARS-induced ARDS; and (iii) determine the therapeutic efficacy of co-administered DAAs and immunomodulators in MERS- and SARS-CoV-infected mice. The impact of these studies is high; investigating new strategies to extend the therapeutic window of DAAs while reversing ARDS disease pathology is broadly relevant to many acute, highly pathogenic respiratory virus infections.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: B2 Project1 CoV AD3C progress report YR1_12-4-19-sm.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

Not Applicable

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

File uploaded: B4 Project1 CoV AD3C progress report YR1_12-4-19-sm.pdf

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

B.6. Plans for the next year.
Our efforts in Year 2 will focus on furthering additional hit compounds through the Go/No Go evaluation scheme. Initial tests will involve potency and activity spectrum. Compounds that meet the Go/No Go criteria described previously will undergo passaging for resistance. In addition, we will continue to determine the mechanism of action and resistance of lead compounds, assess the potential to combine compounds by assessing combined potency and cytotoxicity across a dose matrix, and define the effect of resistance to one drug to efficacy of another drug. We will also further evaluate NHC specificity to viral and not host RNA.

Project 1. The Development of Antivirals for Coronavirus Replication
(Mark R. Denison, Ralph S. Baric)

B.2. What was accomplished in year 1 under the goals of the project?

B.2.a. Major activities, specific objectives, significant results, and key outcomes. Our collaborations with (b) (3) (A), (b) (6) Southern Research (SR), and Emory Institute for Drug Development (EIDD) have been productive in the preclinical development of candidate therapeutics against SARS-CoV, MERS-CoV and potentially pre-pandemic zoonotic CoVs. One key candidate was substantially advanced through analytical pipelines of activity spectrum, *in vivo* efficacy, and mechanisms of antiviral activity and viral resistance.

Hit-to-lead generation of new compounds. We continue to work with our collaborators at EIDD, GS, and SR to enter new screening hits into our compound progression pipeline. SR compounds 35020, 36096, 33684, 36100, 42067, 42300, 42301, 41120, 7958, 43551, and 43552 from their medicinal chemistry group did not exhibit antiviral effects in primary human airway epithelial (HAE) cultures. However, two compounds SR-36097 and SR-35293 showed activity against SARS-CoV (>2 log virus titer reduction at 10 μM) in HAEs. Limited analogues of SR-36097 were synthesized to increase potency and enhance pharmacokinetic properties such as metabolic stability. This series was abandoned due to lack of improvement in antiviral potency and narrow intellectual property. An active analogue, SR-35756, of another series, SR-25293, will be tested in HAEs during this upcoming period along with three new SR hit compounds--36899, 35435, 36099, and 35756. In addition, two new hit compounds, EIDD-2749 and EIDD-2838, inhibit MERS-CoV in HAEs with low micromolar EC_{50} values. We will next test the efficacy and potency of these compounds against other divergent CoVs.

Activity spectrum. We previously demonstrated that both remdesivir (GS-5734) and β-D-N_4-hydroxycytidine (NHC; EIDD-1931) are broad-spectrum inhibitors of our panel of endemic, zoonotic, and pre-zoonotic CoVs. In 2016, a new CoV, swine acute diarrhea syndrome (SADS) CoV, was identified as the causative agent of deadly outbreaks of gastroenteritis in pigs in China. Molecular epidemiology and viral metagenomics suggested this new virus likely emerged from bats. Since this virus is genetically related to human CoV 229E, we sought to determine if it was capable of infecting cells from other host species. We constructed a recombinant SADS-CoV expressing a red fluorescent reporter protein (RFP) and found that SADS-RFP virus was able to infect cell lines from pig, human, non-human primate, and cat. Importantly, SADS-CoV was able to productively infect human primary lung microvascular endothelial cells and fibroblasts. Antiviral assays demonstrated that SADS-CoV was susceptible to remdesivir (EC_{50} = 0.02μM). Next steps include determining the efficacy and potency of NHC in inhibiting SADS-CoV replication.

***In vivo* prophylactic and therapeutic efficacy.** To determine the prophylactic and therapeutic efficacy of NHC against highly pathogenic CoVs, we performed studies in our SARS- and MERS-CoV mouse models of pathogenesis. Our dose-finding studies demonstrated that treatment (P.O., B.I.D.) with 50, 150 or 500mg/kg NHC -2hr prior to infection with either SARS- or MERS-CoV was sufficient to abrogate weight loss as compared to vehicle-treated animals. Given that 50 and 150mg/kg treatment failed to completely prevent SARS-CoV replication, we carried the 500mg/kg dose conditions forward in therapeutic treatment studies. Treatment was initiated -2hr, +12hr, +24hr, and +48hr post-infection to explore the therapeutic time window. With SARS-CoV, mice that received treatment initiated at prior to infection and up to 24hr after infection lost less weight, had improved pulmonary function, and had significantly lower lung viral loads as compared to vehicle. Interestingly, mice that were administered NHC beginning 48hr post-infection did not show appreciable clinical improvement yet had significantly reduced titers in the lung. With MERS-CoV, only treatment initiated at or before 12hr post-infection significantly improved weight loss and pulmonary function, although all treatment groups had significantly reduced viral titers (Fig.1.). Using primer ID,

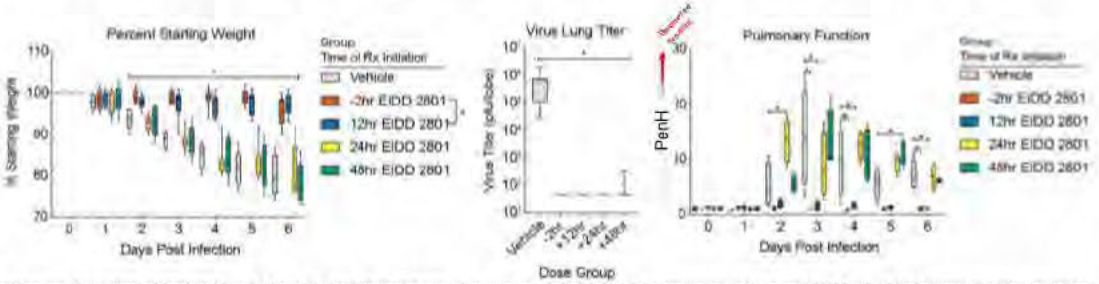


Figure 1. NHC diminishes MERS-CoV disease in mice. 50,000 PFU mouse adapted MERS-CoV was administered intranasally. Mice were treated with 500mg/kg NHC P.O. B.I.D at -2hr, +12hr, +24hr, and +48hr post-infection. Viral titers were reduced in all treatment groups. However, only treatment initiated at or before 12hr post-infection significantly improved weight loss and pulmonary function.

Project 1. The Development of Antivirals for Coronavirus Replication
(Mark R. Denison, Ralph S. Baric)

we analyzed both viral RNA and that of a host gene that is significantly upregulated following MERS-CoV infection (ISG15) to determine if NHC would selectively mutagenize viral RNA in mice. We observed a dose-dependent increase in mutation frequency of viral RNA, but not in the host mRNA ISG15. We also noted an increase in the viral nucleotide transition rate, which was consistent with our cell culture observation. NHC-induced mutations resulted in codon changes, including premature stop codons, in viral RNA. Thus, NHC-mediated mutagenesis was directly correlated with protective efficacy *in vivo*.

Mechanism of action. Our previous data showed that MHV and MERS-CoV acquire a large number of mutations at the consensus level when passaged in the presence of NHC. To probe this phenotype further, we treated WT MHV with increasing concentrations of NHC and performed full-genome next-generation sequencing (NGS) on viral populations released after a single round of infection. The results demonstrated a dose-dependent increase in mutations across the genome. The majority of mutations were G:A and C:U transitions. A dose-dependent increase in C:U mutations was also detected in MERS-CoV genomes produced during treatment of HAEs with a range of NHC doses. When we determined the ratio of infectious virions per viral genome (specific infectivity) released from MHV-infected monolayers treated with NHC, we observed a dose-dependent decrease in specific infectivity. This result indicated that NHC treatment reduces the fraction of infection-competent progeny genomes. To determine if this outcome was attributable to an increased production of defective viral genomes, we quantified genome recombination frequencies at each NHC concentration using a specialized bioinformatics pipeline. Recombination frequency did not change in the presence of NHC, indicating that this compound does not increase defective genome formation. These data further strengthen our evidence that the primary mechanism of NHC anti-CoV activity involves lethal mutagenesis of viral RNA, consistent with NHC mechanism of action described for other viruses, including influenza, IBV, VEEV, and RSV.

Identification of NHC resistance mutations. We passaged MHV and MERS-CoV in NHC-treated cultured cells to assess viral capacity to acquire resistance and gain insights into viral targets of NHC action. By passage 30, one lineage of MHV and two lineages of MERS-CoV displayed low-level (~2-fold) resistance to NHC. Whole-genome sequence analysis revealed a high mutation frequency, with 102 non-synonymous mutations present in P30 MHV and 27 and 41 non-synonymous changes acquired in P30 MERS-CoV. To identify which mutations conferred resistance, we began by engineering mutations in likely targets of NHC, replicase genes, into WT MERS-CoV. These included a V558I change in nsp12, which encodes the RNA-dependent RNA polymerase (RdRp), and two changes in the N7-methyltransferase domain of nsp14. Preliminary data suggest that the triple mutant is less sensitive to NHC compared to WT. Next steps include confirming the phenotype and comparing fitness of NHC-resistant strains to WT MERS-CoV.

Impact of drug resistance on the viral susceptibility spectrum. To assess reciprocal effects of remdesivir and NHC resistance, we treated remdesivir-resistant MHV with increasing doses of NHC and NHC-resistant MHV with increasing doses of remdesivir and quantified viral replication. Our results suggest that remdesivir-resistant MHV is more sensitive to NHC than WT virus, whereas NHC-resistant MHV is equally or marginally more sensitive to remdesivir than WT virus. NHC-resistant MERS-CoV is more sensitive to remdesivir than WT MERS-CoV. Based on these findings, we hypothesize that combination treatment with these compounds may impede resistance development in CoVs.

Combination therapy. We determined whether NHC and remdesivir used in combination alters anti-CoV potency. MHV-infected cells were treated with a dose matrix of NHC and remdesivir in which concentrations of the two compounds were co-varied. A CellTiterGlo cell-viability assay optimized for high-throughput screening was used to determine the effect of drug combinations cytotoxicity and on the cytopathic effect of MHV replication. The results reveal a positive interaction between NHC and remdesivir in the inhibition of MHV replication, while cytotoxicity remained below acceptable levels. These results, in conjunction with absence of detectable cross-resistance, hold promise that NHC and remdesivir could be amendable to use as combination therapy against CoVs.

Project 1. The Development of Antivirals for Coronavirus Replication
(Mark R. Denison, Ralph S. Baric)

B.4. Training Opportunities.
Graduate students are active in the project at Vanderbilt and UNC. Individual development plans (IDPs) are generated on an annual basis for all graduate students. These are used for defining key objectives and goals and reviewed on at least an annual basis. For the AD3C program, IDPs include specific goals relevant to the project. IDPs assist in analysis of progress and future training and career development. Construction of the IDP includes creation, review, and updating of biosketches and CVs; these serve as learning tools for presenting professional training and accomplishments in formats relevant to research funding proposals.

C. COMPONENT PRODUCTS

C.1 PUBLICATIONS

Not Applicable

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Not Applicable

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Not Applicable

C.5 OTHER PRODUCTS AND RESOURCE SHARING

Nothing to report

D. COMPONENT PARTICIPANTS

Not Applicable

E. COMPONENT IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

Not Applicable

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

NOTHING TO REPORT

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?
Not Applicable

F. COMPONENT CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. COMPONENT SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

Not Applicable

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Not Applicable

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT

Not Applicable

G.10 ESTIMATED UNOBLIGATED BALANCE

Not Applicable

G.11 PROGRAM INCOME

Not Applicable

G.12 F&A COSTS

Not Applicable

RPPR - Project-6301

RESEARCH & RELATED BUDGET - SECTION A & B FINAL

ORGANIZATIONAL DUNS*: 6081952770000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL

Start Date*: 03-01-2020 **End Date*:** 02-28-2021

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		[redacted]		(b) (3) (A), (b) (6)	PhD	Site Principal Investigator	(b) (6), (b) (4)	(b) (6)			19,230.00	5,412.00	24,642.00
2.					PhD	Investigator					22,832.00	7,253.00	30,085.00
3.						Investigator					9,615.00	2,706.00	12,321.00
4.						Investigator					22,218.00	7,101.00	29,319.00
5.						Research Associate					22,186.00	7,408.00	29,594.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **125,961.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
1	Graduate Students	(b) (6)			7,875.00	1,717.00	9,592.00
	Undergraduate Students						
	Secretarial/Clerical						
7	1 Project Manager, 2 Investigators, 1 Research Associate, 2 Research Specialist and 1 Research Technician				57,390.00	20,133.00	77,523.00
8	**Total Number Other Personnel**					**Total Other Personnel**	**87,115.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**213,076.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E

ORGANIZATIONAL DUNS*: 6081952770000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL

Start Date*: 03-01-2020 **End Date*:** 02-28-2021

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	7,500.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**7,500.00**

E. Participant/Trainee Support Costs		Funds Requested ($)*
1. Tuition/Fees/Health Insurance		3,450.00
2. Stipends		0.00
3. Travel		0.00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**3,450.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K

ORGANIZATIONAL DUNS*: 6081952770000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL

Start Date*: 03-01-2020 **End Date*:** 02-28-2021

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	92,326.00
2. Publication Costs	2,000.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8. Deep sequencing, histology, flow cytometry, Mi Seq. reactions	34,990.00
9. maintenance, shipping	5,621.00
10. animal per diem	6,000.00
Total Other Direct Costs	**140,937.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**364,963.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. MTDC	55.5	364,963.00	200,640.00
		Total Indirect Costs	**200,640.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number)	Department of Health and Human Services (DHHS) Darryl Mayes (202) 401-2828

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**565,603.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification*	File Name:
	(b) (3) (A) (b)_Budget_Justification_Whitley_CETR_Year_2.pdf
	(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Budget Justification Baric Project Whitley CETR –Year 2

Personnel:

, Ph.D., Principal Investigator (effort, cal months). will supervise the overall direction of the animal research agenda of this highly interactive proposal. He will interact closely with Drs. Denison, to ensure steady progress during the course of the proposal, evaluate results and propose alternative experiments. will be also be responsible for interacting closely with all research staff, holding regular laboratory meetings, communicating research findings with the , writing progress reports and managing fiscal matters associated with the proposal. will work closely together to plan MiSeq based PrimerID experiments planned throughout the proposal. Given the extensive interaction and collaboration with Dr. Denison in the past, he will also lead efforts to coordinate and promote research efforts with the groups. will communicate his findings with Dr. Denison on a regular basis via both conference calls and meetings between the two laboratories.

, Ph.D. Investigator effort, cal months). will oversee the PrimerID work within Project 1 to help define compound mechanism of action. will work with to oversee the work of . He will also work closely with the leadership team to coordinate efforts in his lab with those of the other members of the team. He will be responsible for all reporting for the work done in his lab to and will contribute to the writing of scientific publications covering this work.

TBD, Project Manager/Research Specialist effort, cal months). We are currently searching for a project manager/research specialist to provide administrative and scientific support to this program. This person will help with grants management, budgets and progress reports while also supporting the molecular biology and antiviral assays needed for this endeavor.

, Ph.D. Investigator effort, cal months). has extensive BSL3 experience and is now a Research Assistant Professor within the . He will lead and perform the in vivo drug testing with wild type and resistant mutant strains of MERS-CoV experiments this project. He will be assisted by and . He will consult with and Denison regarding experimental design and analysis and will report findings regularly to the collaborative group as well as interfacing with the drug candidate manufacturers.

, Ph.D. Investigator (effort, cal months). has extensive experience working with wild type SARS-CoV and SARS-ExoN I in mice and is skilled at assembling recombinant SARS-CoV viruses using classic recombinant DNA approaches or synthetic genomic approaches. will lead and perform the in vivo drug testing with wild type and resistant mutant strains of SARS-CoV for this project. will be assisted by and . will consult with and Denison regarding experimental design and analysis and will report findings regularly to the collaborative group as well as interfacing with the drug candidate manufacturers.

, Ph.D. Research Associate effort, cal months). will directly supervise in the creation of deep sequencing libraries. will also be responsible for data analysis, and he will convey the results to other members of the research team. He will contribute, with , in all writing and reporting. has worked over the last years to bring the Primer ID technology into routine practice and is the ideal person to guide and analyze the proposed sequencing work to characterize mutation rates using deep sequencing linked to high accuracy data acquisition.

, Graduate Student effort cal months). is a third year graduate student in the who has recently gained independent access to the BSL3 laboratory. He will work with to determine compound efficacy in SARS-CoV infected mice. He will consult with and regarding experimental design and analysis and will report findings regularly to the collaborative group.

[redacted], **Jr. Research Specialist** [redacted] effort, [redacted] cal months). [redacted] has over [redacted] years of BSL3 experience and pioneered the infectious clone platforms used in the [redacted]. He used synthetic DNAs to construct the SARS-CoV and MERS-CoV molecular clones and isolated numerous recombinant viruses. He is also well versed in performing routine plaque assays and at viral neutralization assays. [redacted] will assist [redacted] with in vivo MERS-CoV drug testing. He will report his results to [redacted] and [redacted] at regular intervals and prepare reports. [redacted] will also be responsible for purchasing supplies, maintaining stocks in the BSL3 and assisting with day to day maintenance of the BSL3 laboratory.

[redacted], **Research Specialist** [redacted] effort, [redacted] cal months). [redacted] has extensive BSL3 experience and will assist with viral titration assays and BSL3 animal infections and husbandry. [redacted] will support [redacted], and [redacted] research efforts as needed and will specifically assist [redacted] with all SARS-CoV in vivo drug testing. [redacted] will also work with [redacted] to maintain BSL3 stocks and in day to day maintenance of the BSL3 laboratory.

[redacted], **Research Technician** [redacted] effort, [redacted] cal months). [redacted] has spent the last 18 months making deep sequencing libraries using the Primer ID technology in the [redacted] thus she is highly skilled to work with [redacted] in generating the data described in this proposal.

Fringe Benefits: Faculty/Staff: 24.863% Social Security and Retirement; $6,306/FTE Health Insurance. Graduate student Research Assistants: 8.99% Social Security and benefits; $4,036/FTE Health Insurance.

Travel

Travel: ($7,500/year): Domestic Travel: Funds are requested for the Project Leader and staff to attend 2 scientific conferences and the annual CETR U19 meeting in Birmingham and the reverse site visits in Washington DC each year. This allows program faculty and fellows to communicate results, develop collaborations and share research interests. International Travel: Funds are requested to allow team members to attend the annual International Conference on Antiviral Research (ICAR) meeting to present current data and renewal and establish collaborations. [redacted] group requested $5,500 in travel funds and [redacted] group $2,000.

Supplies:

Synthetic DNA ($4,000/year) Funds are requested for the purchase of synthetic DNA fragments which are primarily purchased from small biotec companies like Blue Heron or Bio Basic Inc. at costs of about $0.35/base. Our budget allows for ~21,400-bp of synthetic gene synthesis/yr, sufficient for our needs over the course of this project and will allow for the rapid assembly of recombinant viruses bearing different ExoNI derived mutations.

BL3 Protective Gear ($6,000/year) Personnel wear powered air purifying HEPA filtered breathing apparatuses, wear tyvek suits, tyvek aprons, hoods, bocties and are double gloved when entering the BSL3 facility. These materials are expensive as the HEPA, organic chemical filters and even batteries must be replaced every ~6 months, and the tyvek suits are disposable. Moreover, the PAPR (powered air breathing apparatus) are expensive and must be replaced every ~2 years from normal wear and tear, and daily contact with EPA disinfectants. Personnel use high quantities of disinfectants like ethanol, Clorox and other EPA approved disinfectants in maintaining a safe working environment in the BSL3. Personnel spray down tyvek suits, etc. with alcohol or related disinfectants in the process of deconing and leaving the BSL3 facility. All materials that leave the BSL3 must be disinfected, packaged in disinfected, sealed containers, which are disinfected prior to removal from the BSL3 facility. In addition, funds are requested to help defray costs associated with the decontamination and maintenance of the BSL3 laboratory each year.

Miscellaneous ($1,997/year) Monies are requested to purchase glassware, pipettes, etc. used in day to day virologic and cell culture procedures as well as in growing, titering and characterizing virus growth in vitro. Funds are also requested to purchase chemicals, reagents, paper products, gloves, micropipetors, autoclave supplies, plastic tips, water baths, and other small equipment items that typically have short half lives in laboratory settings. [redacted] group requests $1,160 a year and [redacted] group $837 a year.

MiSeq Reagents ($10,829/year) Funds are requested for the (b) (3) (A), (b) (6) to purchase tubes, tips, PCR and indexing reagents to generate five library pools (cDNA synthesis, PCR, primers, indexing kits) to perform Primer ID.

Computer Supplies ($500/year) Funds are requested for project specific computer and software upgrades over the course of the proposal.

Primary Human Lung Cell Cultures ($25,000/year) Funds are requested to purchase mature human airway epithelial cell (HAE) cultures, primary human lung microvascular endothelial cells and fibroblasts for drug testing assays. Each culture is $125. We anticipate requiring 250 HAE cultures annually.

Molecular Biology Reagents ($8,000/year) Assembling recombinant SARS-CoV and MERS-CoV requires large amounts of highly expensive restriction enzymes (e.g., BsmB1, etc.) and large amounts of DNA ligase. In addition, funds are requested for DNA markers, high quality T7 RNA polymerase, and protein and nucleic acid markers. As sequence confirmation is critical prior to assembly of full length genomic cDNA, funds are also requested to sequence modified genomic fragments following introduction of ExoNI mediated mutations.

Immunology Reagents ($4,000/year) Funds to purchase supplies for flow cytometry and ELISA based immune assays are requested to determine how drug treatment/time of addition studies modify the host immune response following CoV infection.

Tissue Culture Reagents ($10,000/year) We are requesting funds to purchase cell culture supplies and plastic ware to perform cell passage and viral plaque assays to determine titer reduction for drug efficacy studies.

Animals ($22,000/year) Funds are requested to purchase ~96 each- SCID ($68), RAG ($124), young BalbC ($25), aged BalbC ($20), young B6 ($20) and aged B6 ($18) mice at the indicated prices per mouse. These monies are essential for evaluating drug efficacy across hosts of differing susceptibilities to lethal infection.

Other Expenses:

Deep Sequencing ($16,600/year) The ExoN mutator phenotype results in high mutation rates which must be accessed by ultra deep sequencing methods like RNAseq, including informatics support to analyze the data. This also includes funds for supplies to generate amplicon library and to prepare the library for sequencing. As such, we anticipate significant sequencing costs over the duration of this proposal. The allotted budget will allow us to work with (b) (3) (A), (b) (6) facility and to be able to sequence approximately 45 samples (~20 samples $10,000) per year.

Publication Costs ($2,000/year) Funds are requested to cover the publication of manuscripts.

Maintenance Contracts ($5,000/year) Several instruments in the (b) (3) (A), (b) (6) that will be used in these studies (centrifuges, CO2-incubators, microscopes) require service contracts for regular maintenance and repairs when needed. These are sophisticated instruments, so the repairs require specialists with appropriate tools and particular replacement parts. A fraction of these costs are included here.

Histology ($7,500/year) Histology slides from paraformaldehyde fixed tissues are prepared on a fee for service basis at UNC Chapel Hill. Given the large number of tissues to be analyzed each year, we are requesting funds to cover this tissue/slide preparation and staining costs.

Flow Cytometry ($3,750/year) (b) (3) (A), (b) (6) provides a core facility with advanced analytical cytometers that can resolve >6 colors at a time, which is needed when delineating subsets of inflammatory cells following infection.

Animal per diem ($6,000/year) SCID, RAG and the young BalbC/B6 animals will be purchased and housed in (b) (3) (A), (b) animal facilities for ~30 days prior to the start of experiments (5 animals per cage x 30 days x 0.62 per cage, $18.6/cage, 10 cages for a typical experiment = $186). The aged BalbC/B6 animals will be purchased and housed in (b) (3) (A), (b) animal facilities for ~90 days prior to the start of experiments (5 animals per cage x 90 days

x 0.62 per cage, $55.8/cage, 20 cages for a typical experiment = $1,116). All MERS-CoV 288/330 mice will be bred in house (~$6,000/year).

MiSeq Deep Sequencing Runs ($7,080/year) Funds are requested for the (b) (3) (A), (b) (6) to perform 5 MiSeq deep sequencing runs per year (300 base paired-end reads) to perform Primer ID.

Shipping ($621/year) Funds are requested to cover the costs of shipping samples/viruses to the (b) (3) (A), (b) (6) for analysis over the course of the proposal.

Tuition ($3,450/year) Funds are requested to cover 25% of (b) (3) (A), (b) (6) tuition costs.

Indirect Costs (Facilities and Administration): In accordance with the University's F&A Cost Rate Agreement dated November 23, 2016, the University's predetermined F&A rate is 55.50%. This rate remains in effect through June 30, 2020. The base for calculating Facilities and Administrative cost (indirect cost) is modified total direct costs (MTDC), consisting of all salaries and wages, fringe benefits, materials and supplies, services, travel and subcontracts up to $25,000 of each subcontract. Modified total direct costs shall exclude equipment, GRA tuition, rental costs of off-site facilities, scholarships, and the portion of each subcontract in excess of $25,000.

A. COMPONENT COVER PAGE

Project Title: Project 2.2: Novel Therapeutics for Emerging Alphavirus

Component Project Lead Information:

Heise, Mark T

B. COMPONENT ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

SPECIFIC AIMS
The goal of this project is the continued development of novel antiviral drugs against emerging alphaviruses. Alphaviruses are arthropod-transmitted RNA viruses comprised of multiple NIAID Category B and C priority pathogens including Venezuelan equine encephalitis (VEEV) and chikungunya (CHIKV) viruses, as well as other re-emerging viruses such as Eastern equine encephalitis (EEEV), Mayaro (MAYV), o'nyong-nyong (ONNV), and Ross River (RRV) viruses. The encephalitic alphaviruses primarily present with neurological disease that can result in high mortality rates in hosts with weakened or immature immune systems, including young and aged populations. Arthritogenic alphaviruses are associated with incapacitating acute and chronic arthralgia, arthritis, and tenosynovitis that can result in long-term disability, and large epidemics with severe economic consequences. Currently, no FDA-licensed vaccines or antiviral therapeutics are available to prevent alphavirus infection or treat disease and thus a critical unmet clinical need exists to combat these viral agents.
In collaboration with Southern Research (SR), we performed high-throughput screen (HTS) campaigns, and confirmatory secondary assays, to identify small molecules that inhibit VEEV and/or CHIKV replication. These screening efforts resulted in the identification of a number of non-toxic lead compounds that effectively block the replication of VEEV, CHIKV, and other alphaviruses. Furthermore, we generated resistant mutants against two highly active compounds and, in doing so, identified the alphavirus nsP2 helicase domain and the nsP3 macrodomain as potential antiviral targets. In addition to this work, collaboration with the Emory Institute for Drug Development (EIDD) identified novel nucleosides, which target RNA-dependent RNA polymerases, with antiviral activity against alphavirus infection in vitro and in vivo. Thus, we have identified potent antiviral compounds against three distinct molecular targets of alphavirus replication.
In this CETR application, we plan to (i) further chemically optimize the lead compounds for antiviral potency, efficacy, and drug-like properties, (ii) define the phylogenetic breadth of antiviral activity, mechanisms of action, and synergism of lead compounds, and (iii) evaluate antiviral activity with single agents and in combination with other leads identified in this project. In addition, we will explore combinations of these antiviral compounds with anti-inflammatory therapies against alphavirus infection in well-characterized pre-clinical animal models of acute and chronic alphavirus disease. To develop novel inhibitors that block alphavirus replication, prevent the emergence of resistance viruses, and reduce disease severity, we propose the following Specific Aims:
Specific Aim 1. To further optimize the inhibitors of alphavirus replication to improve potency and bioavailability. Our previous work resulted in the identification of four chemically distinct leads (SRI-33001, SRI-33394, SRI-34626, and SRI-34963). These leads display potent antiviral activity against diverse alphaviruses with low cytotoxicity. Furthermore, we identified novel nucleoside inhibitors of alphavirus replication, including the lead compound EIDD-1931 and prodrug (EIDD-2801), as well as an active 4'fluorouridine analog EIDD-2749. In this Aim, these lead compounds will be optimized by iterative medicinal chemistry, cell culture based antiviral and cytotoxicity assays, and in vivo pharmacokinetic studies to improve their efficacy, selectivity, solubility, and bioavailability. Optimized compounds will advance to mechanistic (SA2) and pivotal efficacy (SA3) studies.
Specific Aim 2. To determine the antiviral mechanisms of inhibitors of alphavirus replication. In this Aim, we will define the breadth of antiviral activity and cell type specificity, determine the molecular targets through resistance mapping and structural/computational analyses, and identify synergy profiles for active compounds. In preliminary studies, characterization of resistant mutants derived against two highly inhibitory compounds identified the alphavirus nsP2 helicase domain and the nsP3 macrodomain as likely molecular targets. To explore the activity of compounds against these specific viral targets, we have generated a cell line expressing nsP2 helicase and purified nsP2 protein for biochemical/structural assays. Furthermore, we have generated a 1.46 Å resolution crystal structure of the nsP3 macrodomain. We will use this new structural information for in silico computational modeling and empirical structural biology analysis to identify regions of the macrodomain targeted by small molecule inhibitors and their analogs. Finally, we will define the mechanisms of action for our anti-alphavirus compounds and structurally related active analogs that are yet uncharacterized. These studies will inform our compound selections for synergy studies in the context of combination therapy (SA3).
Specific Aim 3. To define the efficacy of mono and combination therapies against alphavirus replication and disease. In this Aim, compounds with optimized antiviral and pharmacokinetic profiles will be evaluated using well-established pre-clinical murine models of alphavirus infection and disease. Given that selection for antiviral drug resistance can adversely impact therapeutic efficacy, and that combination therapies targeting two or more regions of the virus should generate a higher barrier to resistance, new therapeutic regimens will be tested that combine our small molecule inhibitors with novel nucleoside analogs that show robust in vivo efficacy. Lastly, since alphavirus disease is caused in part by virus-induced inflammatory immune pathology, we will evaluate the therapeutic potential of antiviral therapy combined with anti-inflammatory regimens to block both virus replication and virus-induced immune pathology.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: B.2 only Accomplishments Proj 2 2019-sm.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

Not Applicable

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

File uploaded: B.4 Training Opportunities Proj 2-sm.pdf

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

B.6 Plans for next year:

Overview: The goal of this project is to identify novel small molecules capable of inhibiting the replication of diverse members of the Alphavirus genus. We have identified a number of hit molecules with antiviral activity that are at different stages of development. Below is a brief discussion pertaining to each of the compounds that are active in our program and the goals for the upcoming year.

Quinolinones (SR-33394): We are in the process of assembling a manuscript to describe the results from the medicinal chemistry campaign aimed at identifying SAR around this chemical series. Our immediate goals are to perform complementary experiments to identify the breadth of cell types for which SR-33394 compounds are active. We will then finish up writing and submit a manuscript describing these results.

Benzoannulenes (SR-33366): Our immediate goals for this year for this compound series is completion and submission of a manuscript that defines this class of compounds as DHODH inhibitors with activity against a number of RNA viruses including alphaviruses and flaviviruses. In addition, we will perform studies aimed at determining whether combination therapy of this class of inhibitors with other inhibitors including nucleoside analogs will result in antiviral synergism. We hypothesize that combination of DHODH inhibitors with nucleoside analogs may push virus replication into crisis because we will be blocking de novo pyrimidine synthesis and forcing the virus to use the mutating or chain terminating nucleosides. This should enhance the activity of the nucleosides. IF this works then we will try the combination in vivo.

SR-36426: Our immediate goals with this compound series is to continue with the ongoing medicinal chemistry campaign to gain critical information about SAR, to improve solubility and maintain or enhance antiviral activity. We will continue to screen novel compounds based upon this series and attempt to establish a resistant phenotype in order to identify potential mechanisms of action for this series.

SR-33001. Medicinal chemistry will continue to be performed with this compound series to identify compounds with improved pharmacological properties. While an in vivo experiment showed about a two-log reduction in viral loads, this was performed with a compound with solubility issues that were even more apparent in vivo. In addition, at the end of the past year, SR was able to synthesize a biotinylated analog for this series. During the next year we plan to use this compound as a probe to identify the drug target. Thus, our immediate goals are to improve solubility and identify the molecular target(s) for this very interesting chemical series.

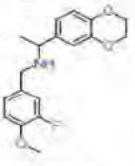
SR-36767. This class of compounds is a first-in-class for CHIKV in that it appears to be a non-nucleoside small molecule inhibitor of nsP4 (viral RNA dependent RNA polymerase). This compound has good antiviral activity in mice. We will continue to perform in vivo efficacy studies for this SR-42718. Immediate goals include performing an experiment to assess whether a higher dosage (80mg/kg; TID) will be sufficient to further reduce tissue viral loads at 7 and 10 days post challenge. Then experiments will be performed that will determine the effect that SR-42718 has on joint pathology as well as to determine the effect of treating at 1 day post infection. The in vivo mouse experiments for SR-42718 should be finished in Year 2. We hope to procure follow-on funding to perform in vivo efficacy experiments in nonhuman primates. In addition, we plan to fully characterize the resistance phenotype that we observed and to increase the breadth of antiviral activity against additional alphaviruses through continued medicinal chemistry studies. We will also initiate structure function studies in order to fully explore nsP4 as a target of this series in order to develop additional small molecules that target nsP4.

Additional Compounds. We validated three compounds including SR-7958, SR-8380, and SR-18457 against a number of alphaviruses. We plan to synthesize and characterize additional nucleoside analogs based upon these scaffolds in order to improve their activity and bioavailability. We will also continue to characterize their molecular action and attempt to generate resistance. Compounds with appropriate activity and bioavailability profiles will be tested in the last part of this upcoming year for efficacy against CHIKV infection in mice.

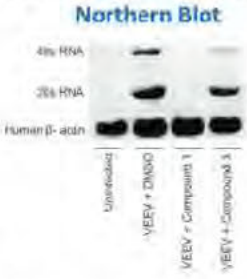
B.2 The goal of this project is to identify novel small molecules capable of inhibiting the replication of diverse members of the Alphavirus genus. Alphaviruses are arthropod-transmitted RNA viruses comprising seven antigenic complexes that include multiple Biodefense Category B and C priority pathogens. Currently, no FDA-licensed vaccines or antiviral therapeutics are available to prevent alphavirus infection or treat disease and thus a critical unmet clinical need exists to combat these viral agents. In collaboration with SR, we performed new high-throughput screen (HTS) campaigns, and confirmatory secondary assays, to identify additional small molecules that inhibit VEEV and/or CHIKV replication. These screening efforts resulted in the identification of a number of non-toxic lead compounds that effectively block the replication of VEEV, CHIKV, and other alphaviruses. During the previous grant period, medicinal chemistry was used to identify more potent, soluble, and stable compounds. Our aim is to chemically optimize alphavirus inhibitors, determine their mechanism of action and breadth and then evaluate the in vivo efficacy of lead compounds. This report summarizes our efforts in 2019 to develop small molecules inhibitors of alphaviruses.

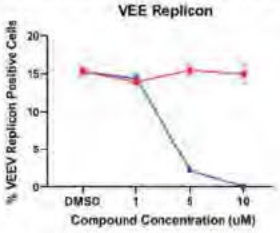
Analysis of compounds from the 2015 VEEV HTS campaign. SR screened 197,025 compounds in a VEEV CPE-based assay in Vero E6 cells and identified 23 compounds for immediate follow-up. OHSU confirmed 8 of 12 active hits including: SR-36426 (IC_{90}=0.72μM), and SR-36427 (IC_{90}=0.25μM).

SRI-36427

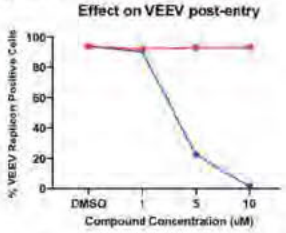


SR-36427. SR generated 59 SR-36427 analogs, but none dramatically improved activity profiles. Analogs SR-38669 (IC_{90}=0.38μM; VLR=9.1 logs at 10μM) and SR-39023 (IC_{90}=0.37μM; VLR=4.88 logs at 10μM) were promising but still had issues of solubility and/or microsomal stability (MLM $t_{1/2}$ = 1.5 minutes). VEEV RNA (northern blot analysis) and protein synthesis were inhibited by SR-36427 indicating that the drug targets nonstructural protein (nsP) functions. SR-36427 blocks VEE replicon activity following infection or transfection, indicating that the compound targets virus replication at a post-entry stage. The generation of resistance was attempted but failed.





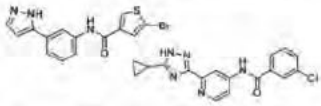
Infection with Replicon



Transfection of Replicon

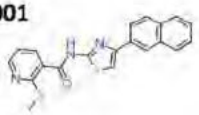
Based upon the inability to generate active analogs with improved stability, SR-36427 has been put on hold. A manuscript describing antiviral activity for the SR-36427 series was accepted for publication in ACS-ID.

SRI-36426 **SRI-42776**

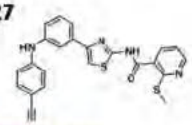


SR-36426. SR generated 75 analogs of SR-36426. The compound displays high activity and low toxicity profiles and some SAR promising analogs display improved activity and solubility. SR-36426 is broadly active against 5 different alphaviruses. SR-36426 inhibits vRNA synthesis and blocks structural protein translation. SR-36426 effectively blocked virus replication in infectious viral RNA transfected cells. *In vivo* pK analysis for SR-42776, a SR-36426 analog, indicated a fast clearance rate ($t_{1/2}$=2.2 hours). SR will continue to synthesize additional analogs for this series to improve pK properties and *in vivo* experiments will be performed on any lead compounds for this series.

SRI-33001

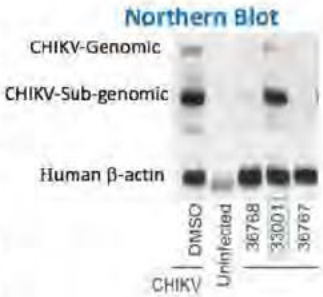


SRI-41627



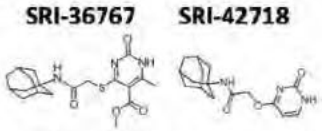
Analysis of compounds from the 2015 CHIKV HTS campaign. SR screened 197,025 compounds in a CHIKV CPE-based assay in Vero E6 cells and identified 26 compounds for immediate follow-up. We confirmed 5 of 11 hits including: SR-33001 (IC_{90}=0.93μM), SR-35756 (IC_{90}=3.39μM), SR-35894 (IC_{90}=0.75μM), and SR-36767 (IC_{90}=0.09μM).

SR-33001. SR generated 90 SR-33001 analogs. SR-41627 (CHIKV-IC_{90}=0.45μM; VLR=8.68 logs at 10μM) was tested for *in vivo* pK activity and found to be devoid of overt toxicity and the compound was cleared with a $t_{1/2}$=6.5hr. SR-33001 is broadly active against alphaviruses, flaviviruses and human cytomegalovirus. SR-33001 does not block viral RNA or nsP protein synthesis but efficiently blocks structural protein synthesis. SR-33001 synergizes with compounds that block RNA synthesis. This profile is similar to our observations with the Src kinase inhibitor Dasatinib (Broeckel *et al.* 2019), which blocks phosphorylation of the kinase S6. SR tested the SR-33001 analog SR-41627 against

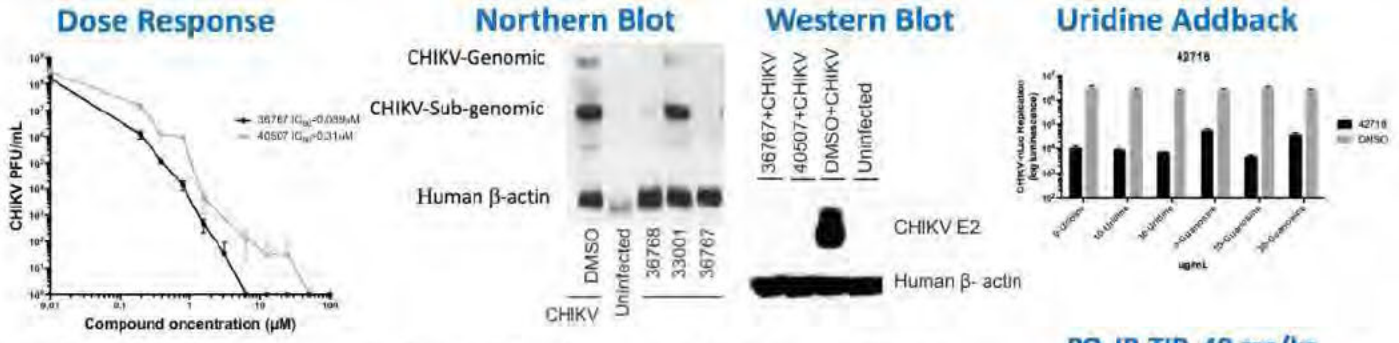




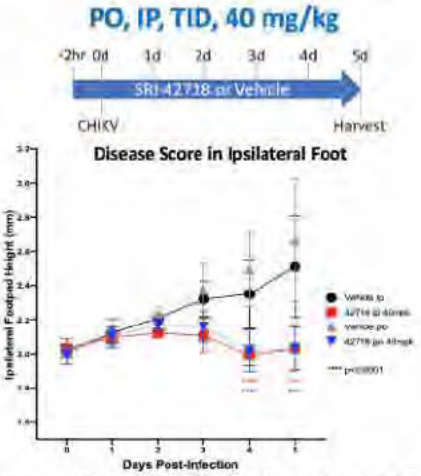
a panel of kinases and OHSU tested SR-33001 for down-regulation of phosphorylation of S6. While Das blocked phosphorylation of S6 in CHIKV-infected cells, this was not observed in cells treated with SR-33001. Collectively, these data suggest that SR-33001 blocks translation of the CHIKV subgenomic vRNA at a step that is different from Dasatinib. Despite extensive attempts, we have been unable to select for resistance against this compound suggestive of a cellular target or an essential viral target that is common among very divergent viruses. In addition, SR-33001 was screened against a limited panel of kinases and no inhibitory activity was observed. In vivo efficacy studies showed little activity, which may be due to poor solubility or permeability for SR-41627. A biotinylated probe has been synthesized for SR-41627 to facilitate target identification studies. Further SAR is aimed at improving solubility, microsomal stability and *in vivo* pK.



SR-36767. SR generated 50 SR-36767 analogs. SR-36767 (IC_{90}=0.089μM; VLR>7 logs at 10μM) is active against many alphaviruses but not flaviviruses. SR-40507 is a pyrimidone analog (IC_{90}=0.31μM; VLR=7.44 logs at 10μM) that was used as a new scaffold to generate SR-42718 (IC_{90}=0.09μM; VLR>7 logs at 10μM), which was recently tested for *in vivo* pK ($t_{1/2}$=6.59hr). The SR-36767 series blocks viral RNA expression (northern blot analysis) and reduces structural protein levels (western blot analysis). DHODH inhibition assays and uridine add-back experiments indicated that the compound was does not target this biosynthetic pathway. Resistance was derived against the analog



SR-40507, which tracks with a mutation in nsP4 (RdRp). Resistance derived against SR-40507 was also active against SR-36767 and SR-42718. Subsequent cloning of the single G to A mutation (Alanine to Threonine) into an infectious clone of CHIKV provided resistance. Preliminary modeling indicates that the mutation is positoned at the inner leaflet of the RNA template tunnel. SR-42718 has a solubility of 62μM in water and decent microsomal stability in mouse, monkey and human microsomes. The compounds is inactive by MiniAmes testing and Cyp activity assays. *In vivo* PK analysis indicates that the compound has good bioavailability by both PO and IP delivery mechanisms but the compound has a short $t_{1/2}$. No adverse toxicity was found by preliminary toxicity studies in mice and the compound was distributed to a number of tissue types including joints and muscles. Importantly, treatment of mice with SR-42718 at 40mg/kg (3x daily; i.p. or p.o.) was sufficient to completely block CHIKV viremia at 3 dpi, reduce joint viral loads by 2-logs at 5 dpi, and reduce joint swelling (from 2-10 dpi) in a mouse model following footpad challenge. BID studies were met with reduced efficacy indicating that TID was required for full activity. In both a 7 & 10 day treatment regimen virus was detected in the ipsilateral joints to near vehicle control levels indicating that either the virus may develop resistance or compound levels dropped below the effective level. Further studies are being designed to improve *in vivo* delivery, bioavailability and efficacy testing. Further SAR is focused on developing compounds with improved pK activity as well as increased breadth of antiviral activity.



Nucleoside Compounds: We validated three nucleoside compounds including SR-7958, SR-8380, and SR-18457 against a number of alphaviruses. SR-7958 and SR-18547 are broadly active against CHIKV, MAYV, ONNV, UNAV, RRV and VEEV. SR-8380 had better activity against CHIKV and UNAV than the other four viruses tested. Time of compound addition studies with CHIKV indicate that all of these compounds must be delivered by 4 hpi in order to achieve antiviral activity. The compounds block viral RNA expression by Northern blot analysis and dramatically reduce PFU/genome ratio. SR has generated analogs and prodrugs of these compounds in an effort to improve efficacy and pK properties for *in vivo* experimentation. We are hoping to perform pK and efficacy testing in 2020 for one or two nucleoside analogs.

Training Plan

[redacted] (Post-Doctoral Fellow-[redacted]

We have designed an individual training plan for [redacted] that will promote [redacted] career development by directly addressing strengths and weaknesses in [redacted] scientific and academic aptitudes. As a postdoctoral fellow [redacted] will have access to the OHSU office for postdoctoral affairs (http://www.ohsu.edu/xd/research/postdocs-students/postdoctoral-fellows-guide/index.cfm). The mission of OHSU's Office of Postdoctoral Affairs (OPA) is to support the career development of all OHSU postdoctoral scholars from arrival to departure. In doing so, the OPA strengthens the research training for all postdoctoral scholars, ensures a consistent and superior postdoctoral experience, and prepares postdoctoral scholars for any professional endeavor they wish to pursue. The most important goal for mentoring students and postdoctoral fellows is to provide them with the skills and experience necessary to be successful at the next level by meeting the following objectives: a) Possess a broad base of established and evolving discipline- and research-specific knowledge and skills; b) Demonstrate effective professional and interpersonal communication skills that allow for interaction with colleagues at all levels; c) Adhere to and reflect professionalism standards and practices within the workplace, institution, and discipline; d) Possess the skills and techniques needed to facilitate effective mentorship, leadership, and management of projects and people; e) Receive training in the responsible conduct of research to improve the ability to make ethical and legal choices; and f) Articulate a plan to pursue a career path of his or [redacted] choosing.

[redacted] presents [redacted] lab work at our formal weekly VGTI meetings, MMI seminars as well as at our combined informal "chalk talk' style lab meetings. [redacted] also attends and presents during our Virology/Immunology Journal Club that meets weekly throughout the year. In addition, we have weekly seminars from outside experts, and post-docs are given opportunities to meet the speakers and discuss their work. Last, [redacted] is expected to present [redacted] research findings at National and International meetings and attend career development meetings provided by OHSU. The VGTI is an excellent training environment for postdoctoral fellows. The opportunities for learning are immense and the combined experience of the faculty is incredible. The VGTI is an amazing environment that provides a great scaffold for learning how to do 'Team Science'.

[redacted] (Post-Doctoral Fellow-[redacted]

An individual training plan is being implemented for [redacted] that promotes [redacted] career development by directly addressing strengths and weaknesses in [redacted] scientific and academic skill sets. All OHSU postdoctoral fellows have access to the OHSU office for postdoctoral affairs (OPA) (http://www.ohsu.edu/xd/research/postdocs-students/postdoctoral-fellows-guide/index.cfm) whose mission is to support career development, research opportunities, and consult on obstacles inherent to postdoctoral training. In these ways OPA strengthens training experience for postdoctoral scholars, facilitates a rewarding postdoctoral experience, and prepares scholars for selecting and pursuing professional endeavors.

Specific goals for postdoctoral training include: 1) Exposure to and refinement of skills needed for experimental inquiry; 2) Support for and emphasis on maintaining a current understanding of rapidly evolving discipline- and topic-specific knowledge; 3) Development of effective interpersonal communication skills that allow for engagement with colleagues at all levels; 4) Understanding adherence to standards of professionalism within the laboratory, institution, and field; 5) Exposure to opportunities that develop effective methods of mentorship, leadership, and project management; 6) Acquire training in the responsible conduct of research to improve make ethical and institutionally compliant choices; and 7) Identification of a career path consistent with the scholar's aspiration.

(b) (6), (b) (3) presents (b) (6), lab work at our formal VGTI meetings once a year as well as at informal work-in-progress style open lab meetings. (b) (6), (b) (3) also attends and presents during a Virology/Immunology Journal Club that meets weekly to stay current in the literature. In addition, VGTI hosts regular seminars from external experts and post-docs are provided opportunities to meet the speakers informally. Lastly, (b) (6), (b) (3) is expected to present (b) (6), research findings at National and International meetings and attend career development meetings provided by OHSU. The VGTI is an excellent training environment for postdoctoral fellows. The opportunities for learning are immense and the combined experience of the faculty is outstanding.

(b) (6), (b) (3) (A)

(b) (6), (b) (3) (A) has worked with (b) (6), (b) (3) (A) for just over two years and we have designed a training program that is specifically tailored to promote (b) (6), (b) (3) (A) career development. (b) (6), (b) (3) (A) has successfully trained seven postdoctoral fellows, all of whom continue to pursue scientific careers in academic, government, or industry related research. Based on this experience, (b) (6), (b) (3) (A) has developed a training program with (b) (6), (b) (3) (A) that is designed to facilitate (b) (6), (b) (3) (A) career development and allow him to meet his long-term goals of becoming an independent investigator. This includes the use of an Individual Development Plan (IDP) designed to facilitate discussion of (b) (6), (b) (3) (A) career goals, while also identifying specific training areas where (b) (6), (b) (3) (A) needs additional development. (b) (6), (b) (3) (A) also has weekly meetings with (b) (6), (b) (3) (A) to discuss scientific progress/plans on his research project, career development, and research ethics. (b) (6), (b) (3) (A) also participates in and presents at a weekly (b) (6), (b) (3) (A) meeting, a weekly Virology in Progress meeting involving (b) (6), (b) (3) virology labs at UNC, and a weekly Department of Genetics Research Colloquium. He also attends weekly research seminar series sponsored by the Departments of Genetics and Microbiology and Immunology, and attends a yearly Virology Colloquium. (b) (6), (b) (3) (A) also attends and presents his research at one or more international research meetings each year.

In addition to the specific training that (b) (6), (b) (3) (A) receives in the (b) (6), (b) (3) (A) lab, as a postdoctoral fellow at UNC, (b) (6), (b) (3) (A) has full access the UNC Office of Postdoctoral Affairs (OPA) https://research.unc.edu › postdoctoral-affairs.
The UNC OPA provides postdoctoral trainees with a comprehensive set of training and support resources. This includes access to career counseling, workshops on career development and communication, and training in Responsible Conduct of Research (RCR). Importantly, (b) (6), (b) (3) (A) is the Faculty Advisor for the UNC OPA, and directly participates in many of OPA's training activities, providing with (b) (6), (b) (3) (A) with additional opportunities to take advantage of the opportunities and resources provided by OPA.

C. COMPONENT PRODUCTS

C.1 PUBLICATIONS

Not Applicable

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Not Applicable

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Not Applicable

C.5 OTHER PRODUCTS AND RESOURCE SHARING

Nothing to report

D. COMPONENT PARTICIPANTS

Not Applicable

E. COMPONENT IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

Not Applicable

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

NOTHING TO REPORT

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?
Not Applicable

F. COMPONENT CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. COMPONENT SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

Not Applicable

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Not Applicable

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT

Not Applicable

G.10 ESTIMATED UNOBLIGATED BALANCE

Not Applicable

G.11 PROGRAM INCOME

Not Applicable

G.12 F&A COSTS

Not Applicable

RESEARCH & RELATED BUDGET - SECTION A & B FINAL

ORGANIZATIONAL DUNS*: 6081952770000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL

Start Date*: 03-01-2020 **End Date*:** 02-28-2021

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		(b) (6), (b) (3) (A)				Project Leader	(b) (6), (b) (4)	(b) (6)			19,230.00	5,412.00	24,642.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **24,642.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	(b) (6)			20,856.00	3,715.00	24,571.00
1	Graduate Students				16,000.00	3,456.00	19,456.00
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Associate				24,453.00	7,972.00	32,425.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**76,452.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**101,094.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E

ORGANIZATIONAL DUNS*: 6081952770000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL

Start Date*: 03-01-2020 **End Date*:** 02-28-2021

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	4,000.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**4,000.00**

E. Participant/Trainee Support Costs		Funds Requested ($)*
1. Tuition/Fees/Health Insurance		3,000.00
2. Stipends		0.00
3. Travel		0.00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**3,000.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K

ORGANIZATIONAL DUNS*: 6081952770000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL

Start Date*: 03-01-2020 **End Date*:** 02-28-2021

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	47,477.00
2. Publication Costs	2,000.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8. Histology	5,000.00
9. Service Contracts	4,500.00
Total Other Direct Costs	**58,977.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**167,071.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. MDTC	55.5	164,071.00	91,059.00
		Total Indirect Costs	**91,059.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Darryl Mayes, 202-401-2808

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**258,130.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification* File Name: (b) (6), (b) (3) Justification AD3C Yr 2 mth (11-2019).pdf

(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

BUDGET JUSTIFICATION

Personnel:

(b) (6), (b) (3) (A), **Ph.D., Co-Investigator** ((b) (6) Calendar Months, (b) (6) effort). (b) (6), (b) (3) (A) . He will be responsible for the overseeing the testing of candidate therapeutics for their ability to protect mice from acute CHIKV induced arthritis or VEE-induced viral encephalitis. He will work in close collaboration with (b) (6), (b) (3) (A) and Streblow, as well as the other research project leaders to set priorities for which drugs should be tested within the CHIKV or VEE models and to identify promising candidates that show promising in vivo potential so that those candidates can be taken forward for further optimization.

(b) (6), (b) (3) (A) **Ph.D. Postdoctoral Fellow** ((b) (6) Calendar Months, (b) (6) effort). (b) (6), (b) (3) (A) He will administer therapeutics, perform CHIKV and VEE infections, and will monitor infected animals for disease signs and collect tissues to assess viral loads and virus-induced pathology. He also directs the day to day operations of the BSL-3 facility where all work with CHIKV and VEE is conducted and will oversee the proper training and compliance of all individuals working within the BSL-3 facility.

(b) (6), (b) (3) (A) **Ph.D. Research Associate** ((b) (6) Calendar Months, (b) (6) effort). (b) (6), (b) (3) (A) will be responsible for coordinating in vivo mouse studies and will be involved in the administration of candidate therapies and viral challenge studies. He will also assist in collection of data to assess the impact of therapeutics on viral loads, virus-induced disease, and virus induced pathology within joint (CHIKV) and the central nervous system (VEE).

(b) (6), (b) (3) (A) **Graduate Research Assistant** ((b) (6) Calendar Months, (b) (6) effort). (b) (6), (b) (3) (A) to provide assistance in viral challenges, analysis of virus-induced disease phenotypes, and quantification of viral loads in the drug protection studies.

Fringe Benefits: Faculty/Staff: 24.863% Social Security and Retirement; $6306/FTE Health Insurance. Postdoctoral fellow benefits are calculated at 8.99% of salary and $4600/FTE for health insurance. GRA benefits are calculated 8.9% and $4,036 for health insurance.

SUPPLIES ($47,477):

The evaluation of candidate therapies against either CHIKV or VEE requires the assessment of viral loads, evaluation of inflammatory cell infiltration and pathology within these tissues. Therefore, funds are requested to cover the costs of tissue culture consumables (plastic ware, media, serum) required for the assessment of viral loads within the joints or CNS. We will also need to generate viral stocks, as well as generate infectious clones containing potential escape mutants and funds are requested to cover the costs of the molecular biology supplies needed for those purposes. We are also requesting funds to cover the cost of purchasing adult C57Bl/6 mice, which will be used for testing candidate therapies against both CHIKV and VEE, as well as funds to cover the cost of supplies needed to house these animals within our BSL-3 laboratory. Lastly, since some assays will need to be performed under BSL-3 conditions, funds are requested to cover the cost of personal protective gears, such as gloves, tyvek suits, and PAPRs.

TRAVEL ($4,000):

Funds are requested for the Project Leader, and 2 lab members to attend 1 scientific meeting to present findings and interact with other scientists in the field and to attend programmatic meetings.

OTHER EXPENSES ($14,500):

Equipment service contracts ($4,500): Several instruments in the Heise Laboratory that will be used in these studies (4deg centrifuge, CO2-incubators, microscopes) require service contracts for regular maintenance and repairs when needed. These are sophisticated instruments, so the repairs require specialists with appropriate tools and particular replacement parts. A fraction of these costs are included here.

Histology Costs ($5,000) Histology slides from paraformaldehyde fixed tissues are prepared on a fee for service basis at UNC. Given the large number of tissues to be analyzed each year, we are requesting funds to cover this tissue/slide preparation and staining costs.

Publication Costs ($2,000) Funds are requested to cover the cost of publishing scientific results from this project.

Tuition ($3,000) As per University policy, tuition is budgeted based on student effort.

Federal F&A Cost Rate: In accordance to an agreement between DHHS and UNC dated November 23, 2016, the indirect cost rate is 55.5% of modified total direct costs.

A. COMPONENT COVER PAGE

Project Title: Project 2.3: Novel Therapeutics for Emerging Alphavirus

Component Project Lead Information:

(b) (6), (b) (3) (A)

B. COMPONENT ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

SPECIFIC AIMS
The goal of this project is the continued development of novel antiviral drugs against emerging alphaviruses. Alphaviruses are arthropod-transmitted RNA viruses comprised of multiple NIAID Category B and C priority pathogens including Venezuelan equine encephalitis (VEEV) and chikungunya (CHIKV) viruses, as well as other re-emerging viruses such as Eastern equine encephalitis (EEEV), Mayaro (MAYV), o'nyong-nyong (ONNV), and Ross River (RRV) viruses. The encephalitic alphaviruses primarily present with neurological disease that can result in high mortality rates in hosts with weakened or immature immune systems, including young and aged populations. Arthritogenic alphaviruses are associated with incapacitating acute and chronic arthralgia, arthritis, and tenosynovitis that can result in long-term disability, and large epidemics with severe economic consequences. Currently, no FDA-licensed vaccines or antiviral therapeutics are available to prevent alphavirus infection or treat disease and thus a critical unmet clinical need exists to combat these viral agents.
In collaboration with Southern Research (SR), we performed high-throughput screen (HTS) campaigns, and confirmatory secondary assays, to identify small molecules that inhibit VEEV and/or CHIKV replication. These screening efforts resulted in the identification of a number of non-toxic lead compounds that effectively block the replication of VEEV, CHIKV, and other alphaviruses. Furthermore, we generated resistant mutants against two highly active compounds and, in doing so, identified the alphavirus nsP2 helicase domain and the nsP3 macrodomain as potential antiviral targets. In addition to this work, collaboration with the Emory Institute for Drug Development (EIDD) identified novel nucleosides, which target RNA-dependent RNA polymerases, with antiviral activity against alphavirus infection in vitro and in vivo. Thus, we have identified potent antiviral compounds against three distinct molecular targets of alphavirus replication.
In this CETR application, we plan to (i) further chemically optimize the lead compounds for antiviral potency, efficacy, and drug-like properties, (ii) define the phylogenetic breadth of antiviral activity, mechanisms of action, and synergism of lead compounds, and (iii) evaluate antiviral activity with single agents and in combination with other leads identified in this project. In addition, we will explore combinations of these antiviral compounds with anti-inflammatory therapies against alphavirus infection in well-characterized pre-clinical animal models of acute and chronic alphavirus disease. To develop novel inhibitors that block alphavirus replication, prevent the emergence of resistance viruses, and reduce disease severity, we propose the following Specific Aims:
Specific Aim 1. To further optimize the inhibitors of alphavirus replication to improve potency and bioavailability. Our previous work resulted in the identification of four chemically distinct leads (SRI-33001, SRI-33394, SRI-34626, and SRI-34963). These leads display potent antiviral activity against diverse alphaviruses with low cytotoxicity. Furthermore, we identified novel nucleoside inhibitors of alphavirus replication, including the lead compound EIDD-1931 and prodrug (EIDD-2801), as well as an active 4'fluorouridine analog EIDD-2749. In this Aim, these lead compounds will be optimized by iterative medicinal chemistry, cell culture based antiviral and cytotoxicity assays, and in vivo pharmacokinetic studies to improve their efficacy, selectivity, solubility, and bioavailability. Optimized compounds will advance to mechanistic (SA2) and pivotal efficacy (SA3) studies.
Specific Aim 2. To determine the antiviral mechanisms of inhibitors of alphavirus replication. In this Aim, we will define the breadth of antiviral activity and cell type specificity, determine the molecular targets through resistance mapping and structural/computational analyses, and identify synergy profiles for active compounds. In preliminary studies, characterization of resistant mutants derived against two highly inhibitory compounds identified the alphavirus nsP2 helicase domain and the nsP3 macrodomain as likely molecular targets. To explore the activity of compounds against these specific viral targets, we have generated a cell line expressing nsP2 helicase and purified nsP2 protein for biochemical/structural assays. Furthermore, we have generated a 1.46 Å resolution crystal structure of the nsP3 macrodomain. We will use this new structural information for in silico computational modeling and empirical structural biology analysis to identify regions of the macrodomain targeted by small molecule inhibitors and their analogs. Finally, we will define the mechanisms of action for our anti-alphavirus compounds and structurally related active analogs that are yet uncharacterized. These studies will inform our compound selections for synergy studies in the context of combination therapy (SA3).
Specific Aim 3. To define the efficacy of mono and combination therapies against alphavirus replication and disease. In this Aim, compounds with optimized antiviral and pharmacokinetic profiles will be evaluated using well-established pre-clinical murine models of alphavirus infection and disease. Given that selection for antiviral drug resistance can adversely impact therapeutic efficacy, and that combination therapies targeting two or more regions of the virus should generate a higher barrier to resistance, new therapeutic regimens will be tested that combine our small molecule inhibitors with novel nucleoside analogs that show robust in vivo efficacy. Lastly, since alphavirus disease is caused in part by virus-induced inflammatory immune pathology, we will evaluate the therapeutic potential of antiviral therapy combined with anti-inflammatory regimens to block both virus replication and virus-induced immune pathology.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: B.2 only Accomplishments Proj 2 2019-sm.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

Not Applicable

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

NOTHING TO REPORT

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

B.6 Plans for next year:

Overview: The goal of this project is to identify novel small molecules capable of inhibiting the replication of diverse members of the Alphavirus genus. We have identified a number of hit molecules with antiviral activity that are at different stages of development. Below is a brief discussion pertaining to each of the compounds that are active in our program and the goals for the upcoming year.

Quinolinones (SR-33394): We are in the process of assembling a manuscript to describe the results from the medicinal chemistry campaign aimed at identifying SAR around this chemical series. Our immediate goals are to perform complementary experiments to identify the breadth of cell types for which SR-33394 compounds are active. We will then finish up writing and submit a manuscript describing these results.

Benzoannulenes (SR-33366): Our immediate goals for this year for this compound series is completion and submission of a manuscript that defines this class of compounds as DHODH inhibitors with activity against a number of RNA viruses including alphaviruses and flaviviruses. In addition, we will perform studies aimed at determining whether combination therapy of this class of inhibitors with other inhibitors including nucleoside analogs will result in antiviral synergism. We hypothesize that combination of DHODH inhibitors with nucleoside analogs may push virus replication into crisis because we will be blocking de novo pyrimidine synthesis and forcing the virus to use the mutating or chain terminating nucleosides. This should enhance the activity of the nucleosides. IF this works then we will try the combination in vivo.
SR-36426: Our immediate goals with this compound series is to continue with the ongoing medicinal chemistry campaign to gain critical information about SAR, to improve solubility and maintain or enhance antiviral activity. We will continue to screen novel compounds based upon this series and attempt to establish a resistant phenotype in order to identify potential mechanisms of action for this series.

SR-33001. Medicinal chemistry will continue to be performed with this compound series to identify compounds with improved pharmacological properties. While an in vivo experiment showed about a two-log reduction in viral loads, this was performed with a compound with solubility issues that were even more apparent in vivo. In addition, at the end of the past year, SR was able to synthesize a biotinylated analog for this series. During the next year we plan to use this compound as a probe to identify the drug target. Thus, our immediate goals are to improve solubility and identify the molecular target(s) for this very interesting chemical series.

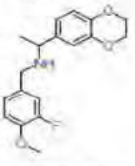
SR-36767. This class of compounds is a first-in-class for CHIKV in that it appears to be a non-nucleoside small molecule inhibitor of nsP4 (viral RNA dependent RNA polymerase). This compound has good antiviral activity in mice. We will continue to perform in vivo efficacy studies for this SR-42718. Immediate goals include performing an experiment to assess whether a higher dosage (80mg/kg; TID) will be sufficient to further reduce tissue viral loads at 7 and 10 days post challenge. Then experiments will be performed that will determine the effect that SR-42718 has on joint pathology as well as to determine the effect of treating at 1 day post infection. The in vivo mouse experiments for SR-42718 should be finished in Year 2. We hope to procure follow-on funding to perform in vivo efficacy experiments in nonhuman primates. In addition, we plan to fully characterize the resistance phenotype that we observed and to increase the breadth of antiviral activity against additional alphaviruses through continued medicinal chemistry studies. We will also initiate structure function studies in order to fully explore nsP4 as a target of this series in order to develop additional small molecules that target nsP4.

Additional Compounds. We validated three compounds including SR-7958, SR-8380, and SR-18457 against a number of alphaviruses. We plan to synthesize and characterize additional nucleoside analogs based upon these scaffolds in order to improve their activity and bioavailability. We will also continue to characterize their molecular action and attempt to generate resistance. Compounds with appropriate activity and bioavailability profiles will be tested in the last part of this upcoming year for efficacy against CHIKV infection in mice.

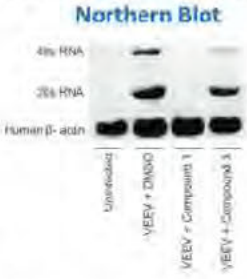
B.2 The goal of this project is to identify novel small molecules capable of inhibiting the replication of diverse members of the Alphavirus genus. Alphaviruses are arthropod-transmitted RNA viruses comprising seven antigenic complexes that include multiple Biodefense Category B and C priority pathogens. Currently, no FDA-licensed vaccines or antiviral therapeutics are available to prevent alphavirus infection or treat disease and thus a critical unmet clinical need exists to combat these viral agents. In collaboration with SR, we performed new high-throughput screen (HTS) campaigns, and confirmatory secondary assays, to identify additional small molecules that inhibit VEEV and/or CHIKV replication. These screening efforts resulted in the identification of a number of non-toxic lead compounds that effectively block the replication of VEEV, CHIKV, and other alphaviruses. During the previous grant period, medicinal chemistry was used to identify more potent, soluble, and stable compounds. Our aim is to chemically optimize alphavirus inhibitors, determine their mechanism of action and breadth and then evaluate the in vivo efficacy of lead compounds. This report summarizes our efforts in 2019 to develop small molecules inhibitors of alphaviruses.

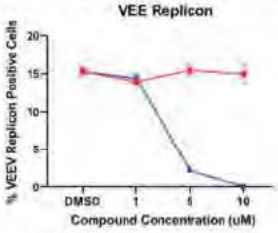
Analysis of compounds from the 2015 VEEV HTS campaign. SR screened 197,025 compounds in a VEEV CPE-based assay in Vero E6 cells and identified 23 compounds for immediate follow-up. OHSU confirmed 8 of 12 active hits including: SR-36426 (IC_{90}=0.72μM), and SR-36427 (IC_{90}=0.25μM).

SRI-36427

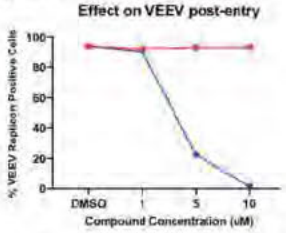


SR-36427. SR generated 59 SR-36427 analogs, but none dramatically improved activity profiles. Analogs SR-38669 (IC_{90}=0.38μM; VLR=9.1 logs at 10μM) and SR-39023 (IC_{90}=0.37μM; VLR=4.88 logs at 10μM) were promising but still had issues of solubility and/or microsomal stability (MLM $t_{1/2}$ = 1.5 minutes). VEEV RNA (northern blot analysis) and protein synthesis were inhibited by SR-36427 indicating that the drug targets nonstructural protein (nsP) functions. SR-36427 blocks VEE replicon activity following infection or transfection, indicating that the compound targets virus replication at a post-entry stage. The generation of resistance was attempted but failed.





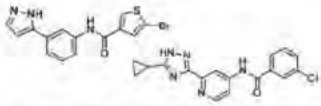
Infection with Replicon



Transfection of Replicon

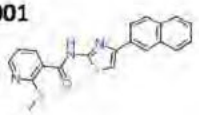
Based upon the inability to generate active analogs with improved stability, SR-36427 has been put on hold. A manuscript describing antiviral activity for the SR-36427 series was accepted for publication in ACS-ID.

SRI-36426 **SRI-42776**



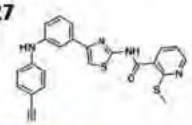
SR-36426. SR generated 75 analogs of SR-36426. The compound displays high activity and low toxicity profiles and some SAR promising analogs display improved activity and solubility. SR-36426 is broadly active against 5 different alphaviruses. SR-36426 inhibits vRNA synthesis and blocks structural protein translation. SR-36426 effectively blocked virus replication in infectious viral RNA transfected cells. *In vivo* pK analysis for SR-42776, a SR-36426 analog, indicated a fast clearance rate ($t_{1/2}$=2.2 hours). SR will continue to synthesize additional analogs for this series to improve pK properties and *in vivo* experiments will be performed on any lead compounds for this series.

SRI-33001

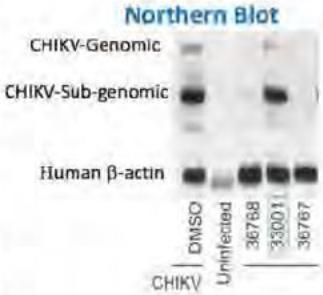


Analysis of compounds from the 2015 CHIKV HTS campaign. SR screened 197,025 compounds in a CHIKV CPE-based assay in Vero E6 cells and identified 26 compounds for immediate follow-up. We confirmed 5 of 11 hits including: SR-33001 (IC_{90}=0.93μM), SR-35756 (IC_{90}=3.39μM), SR-35894 (IC_{90}=0.75μM), and SR-36767 (IC_{90}=0.09μM).

SRI-41627



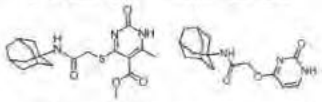
SR-33001. SR generated 90 SR-33001 analogs. SR-41627 (CHIKV-IC_{90}=0.45μM; VLR=8.68 logs at 10μM) was tested for *in vivo* pK activity and found to be devoid of overt toxicity and the compound was cleared with a $t_{1/2}$=6.5hr. SR-33001 is broadly active against alphaviruses, flaviviruses and human cytomegalovirus. SR-33001 does not block viral RNA or nsP protein synthesis but efficiently blocks structural protein synthesis. SR-33001 synergizes with compounds that block RNA synthesis. This profile is similar to our observations with the Src kinase inhibitor Dasatinib (Broeckel *et al.* 2019), which blocks phosphorylation of the kinase S6. SR tested the SR-33001 analog SR-41627 against



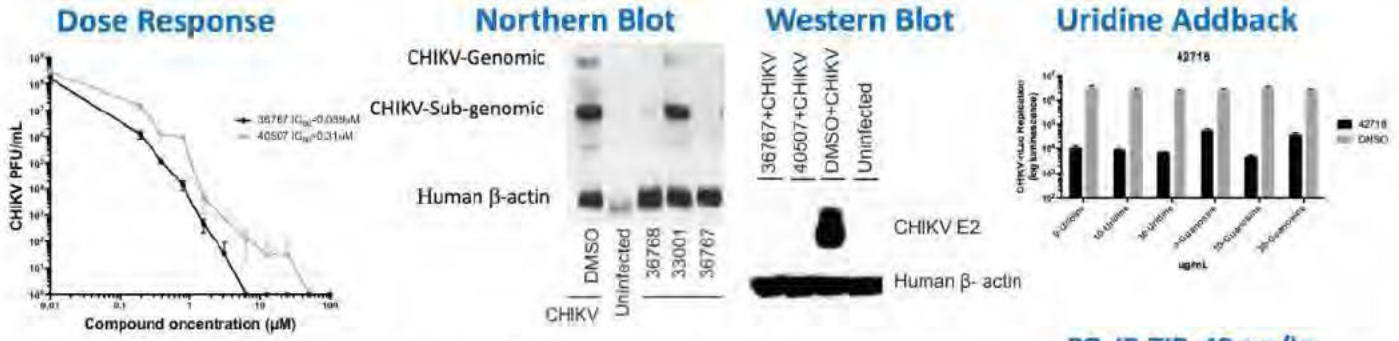


a panel of kinases and OHSU tested SR-33001 for down-regulation of phosphorylation of S6. While Das blocked phosphorylation of S6 in CHIKV-infected cells, this was not observed in cells treated with SR-33001. Collectively, these data suggest that SR-33001 blocks translation of the CHIKV subgenomic vRNA at a step that is different from Dasatinib. Despite extensive attempts, we have been unable to select for resistance against this compound suggestive of a cellular target or an essential viral target that is common among very divergent viruses. In addition, SR-33001 was screened against a limited panel of kinases and no inhibitory activity was observed. In vivo efficacy studies showed little activity, which may be due to poor solubility or permeability for SR-41627. A biotinylated probe has been synthesized for SR-41627 to facilitate target identification studies. Further SAR is aimed at improving solubility, microsomal stability and *in vivo* pK.

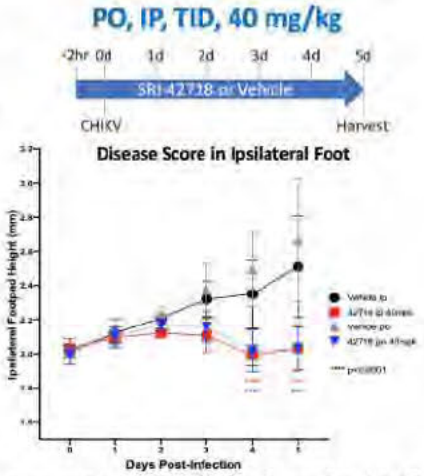
SRI-36767 **SRI-42718**



SR-36767. SR generated 50 SR-36767 analogs. SR-36767 (IC_{90}=0.089μM; VLR>7 logs at 10μM) is active against many alphaviruses but not flaviviruses. SR-40507 is a pyrimidone analog (IC_{90}=0.31μM; VLR=7.44 logs at 10μM) that was used as a new scaffold to generate SR-42718 (IC_{90}=0.09μM; VLR>7 logs at 10μM), which was recently tested for *in vivo* pK ($t_{1/2}$=6.59hr). The SR-36767 series blocks viral RNA expression (northern blot analysis) and reduces structural protein levels (western blot analysis). DHODH inhibition assays and uridine add-back experiments indicated that the compound was does not target this biosynthetic pathway. Resistance was derived against the analog



SR-40507, which tracks with a mutation in nsP4 (RdRp). Resistance derived against SR-40507 was also active against SR-36767 and SR-42718. Subsequent cloning of the single G to A mutation (Alanine to Threonine) into an infectious clone of CHIKV provided resistance. Preliminary modeling indicates that the mutation is positoned at the inner leaflet of the RNA template tunnel. SR-42718 has a solubility of 62μM in water and decent microsomal stability in mouse, monkey and human microsomes. The compounds is inactive by MiniAmes testing and Cyp activity assays. *In vivo* PK analysis indicates that the compound has good bioavailability by both PO and IP delivery mechanisms but the compound has a short $t_{1/2}$. No adverse toxicity was found by preliminary toxicity studies in mice and the compound was distributed to a number of tissue types including joints and muscles. Importantly, treatment of mice with SR-42718 at 40mg/kg (3x daily; i.p. or p.o.) was sufficient to completely block CHIKV viremia at 3 dpi, reduce joint viral loads by 2-logs at 5 dpi, and reduce joint swelling (from 2-10 dpi) in a mouse model following footpad challenge. BID studies were met with reduced efficacy indicating that TID was required for full activity. In both a 7 & 10 day treatment regimen virus was detected in the ipsilateral joints to near vehicle control levels indicating that either the virus may develop resistance or compound levels dropped below the effective level. Further studies are being designed to improve *in vivo* delivery, bioavailability and efficacy testing. Further SAR is focused on developing compounds with improved pK activity as well as increased breadth of antiviral activity.



Nucleoside Compounds: We validated three nucleoside compounds including SR-7958, SR-8380, and SR-18457 against a number of alphaviruses. SR-7958 and SR-18547 are broadly active against CHIKV, MAYV, ONNV, UNAV, RRV and VEEV. SR-8380 had better activity against CHIKV and UNAV than the other four viruses tested. Time of compound addition studies with CHIKV indicate that all of these compounds must be delivered by 4 hpi in order to achieve antiviral activity. The compounds block viral RNA expression by Northern blot analysis and dramatically reduce PFU/genome ratio. SR has generated analogs and prodrugs of these compounds in an effort to improve efficacy and pK properties for *in vivo* experimentation. We are hoping to perform pK and efficacy testing in 2020 for one or two nucleoside analogs.

C. COMPONENT PRODUCTS

C.1 PUBLICATIONS

Not Applicable

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Not Applicable

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Not Applicable

C.5 OTHER PRODUCTS AND RESOURCE SHARING

Nothing to report

D. COMPONENT PARTICIPANTS

Not Applicable

E. COMPONENT IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

Not Applicable

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

NOTHING TO REPORT

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?
Not Applicable

F. COMPONENT CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. COMPONENT SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

Not Applicable

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Not Applicable

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT

Not Applicable

G.10 ESTIMATED UNOBLIGATED BALANCE

Not Applicable

G.11 PROGRAM INCOME

Not Applicable

G.12 F&A COSTS

Not Applicable

RESEARCH & RELATED BUDGET - SECTION A & B FINAL

ORGANIZATIONAL DUNS*: 0410963140000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF COLORADO AT DENVER

Start Date*: 03-01-2020 **End Date*:** 02-28-2021

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1. Dr	(b) (6), (b) (3) (A)				Site Principal Investigator	(b) (6), (b) (4)	(b) (6)			11,793.00	4,339.00	16,132.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **16,132.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	(b) (6)			25,002.00	6,358.00	31,360.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Technician				22,553.00	9,254.00	31,807.00
2	**Total Number Other Personnel**					**Total Other Personnel**	**63,167.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**79,299.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E

ORGANIZATIONAL DUNS*: 0410963140000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF COLORADO AT DENVER

Start Date*: 03-01-2020 **End Date*:** 02-28-2021

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		0.00
2. Stipends		0.00
3. Travel		0.00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K

ORGANIZATIONAL DUNS*: 0410963140000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF COLORADO AT DENVER

Start Date*: 03-01-2020 **End Date*:** 02-28-2021

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	49,260.00
2. Publication Costs	2,000.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8. Animal Costs	26,500.00
Total Other Direct Costs	**77,760.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**160,059.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. MTDC	55.5	160,060.00	88,833.00
		Total Indirect Costs	**88,833.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Darryl Mayes, 202-401-2808

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**248,892.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification* File Name: 12.04.2019 UAB_BudgetJust Y2-UnivCO.pdf

(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

BUDGET JUSTIFICATION

(b) (6), (b) (3) (A)

University of Colorado School of Medicine

PERSONNEL ($79,299):

(b) (6), (b) (3) (A) **Ph.D., Principal Investigator.** (b) (6), (b) (3) (A) will devote (b) (6) calendar months to this project and receive (b) (6) calendar months of salary support. (b) (6), (b) (3) (A) has extensive experience and expertise in alphavirus pathogenesis, host responses to virus infection, and mouse models of infectious diseases. (b) (6), (b) (3) (A) will be responsible for the design and oversight of all investigations occurring in his laboratory. He will actively participate in the planning and execution of experiments, as well as in the generation of progress reports and manuscripts.

(b) (6), (b) (3) (A), **Professional Research Assistant.** (b) (6), (b) (3) (A) will devote (b) (6) calendar months of time to this project and derive (b) (6) calendar months of salary from it. (b) (6), (b) (3) (A) will assist with the general management of this project. He will assist with management of the mouse colony, tissue culture experiments, generation of stocks of infectious viruses for assays, RNA isolations, and PCR analysis. (b) (6), (b) (3) (A) also will be responsible for general lab management, inventory, and reagent/supply orders.

TBD, Postdoctoral Researcher. A postdoctoral researcher will be recruited for this project. This person will devote 6 calendar months of time to this project and derive 6 calendar months salary from it. This person will perform tissue culture experiments to define mechanisms of resistance to antiviral compounds and animal infection experiments to evaluate combination anti-viral plus anti-inflammatory therapeutics. This person also will assist with data analysis, data interpretation, and manuscript preparation.

SUPPLIES Y2: $49,260:

Tissue culture. The funds requested will be used for media preparation, serum, antibiotics, and plasticware (disposable pipets, pipetman tips, flasks, tubes, cryogenic vials, filtration flasks, sterile bottles).

Molecular Biology Reagents. Funds are requested to cover the costs of consumables such as Trizol, RNA isolation kits, reverse-transcriptase enzyme, and PCR reagents. These reagents will be used for quantification of CHIKV RNA in tissues and cells via quantitative real-time RT-PCR. We also request funds for antibodies and consumables used to evaluate viral titers by plaque assays or focus-forming assays In addition, the performance of these studies requires stocks of infectious CHIKV, VEEV, and other alphaviruses generated from cDNA clones. Therefore, we request funds for restriction enzymes, plasmid DNA purification kits, bacterial culture supplies, DNA/RNA electrophoresis, and *in vitro* transcription kits.

General. We request funds to cover the costs associated with the generation of fixed frozen tissue sections ($5 per section), paraffin-embedded tissue sections ($6.50 per section), and staining of tissue sections with hematoxylin and eosin or other special stains for the evaluation of histopathological changes ($3.25 per section). This amount also includes funds for liquid N2/CO2 bottled gas and BSL3 biosafety laboratory operation (personal protective equipment and BSL3 waste disposal).

OTHER EXPENSES:

Animals (Y2: $26,500):

Animal purchase. The acute and chronic CHIKV infection model and the VEEV model utilize WT C57BL/6 mice. Therefore, we request funds to cover costs associated with the purchase WT C57BL/6 mice ($22/mouse; JAX mice). CHIKV and VEEV infection studies also may use genetically altered mice that display enhanced sensitivity to infection.

Animal breeding. Funds are requested to maintain breeders of WT and genetically altered strains of C57BL/6 mice in the AMC mouse facility ($0.92 per cage per day).

Animal housing. Funds are requested for cage charges for all mice immediately before and after infection with VEEV or CHIKV, BSL2 and BSL3 pathogens depending on the strain used. All infection experiments will take place in the ABSL2 or ABSL3, which has higher per diems ($1.11 per cage).

Travel ($3,000):

Travel is requested for (b) (6), (b) (3) (A) and the postdoctoral researcher working on the project to attend one meeting per year for presentation of scientific findings.

Publication Costs ($2,000):

Funds are budgeted for publication costs.

Specialized Costs associated with BSL3. In comparison to standard BSL2 work, additional costs are incurred for BSL3 and ABSL3 work. These include user fees for the BSL3 that are paid to the institution to offset the cost of the facility, the purchase and utilization of personal protection equipment (PPE) for every experiment, biosafety waste management, and higher animal facility cost. **Given the extensive experiments conducted under BSL3 conditions with pathogenic strains of CHIKV, our costs are necessarily greater than standard BSL2 work**.

A. COMPONENT COVER PAGE

Project Title: Project 3.3: Develop Antiviral Agents for Flavivirus Therapy
Component Project Lead Information: (b) (6), (b) (3) (A)

B. COMPONENT ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

SPECIFIC AIMS. The flavivirus genus comprises over 70 positive, single-stranded RNA viruses. Many flaviviruses are significant human pathogens, among which dengue virus (DENV) is the most important mosquito-transmitted virus with over 390 million infections per year. West Nile virus (WNV), yellow fever virus (YFV), Japanese encephalitis virus (JEV), tick-borne encephalitis virus (TBEV), and recently Zika virus (ZIKV) also cause global outbreaks and epidemics, posing constant threats to public health1. No FDA-approved antiviral drug is available to treat any flavivirus infection, and vaccines are lacking against several family members. Thus, the development of flavivirus antiviral therapy is a public health priority.

The goal of this proposal is to develop direct antiviral agents (DAAs) for flavivirus therapy. Antiviral development for all RNA viruses, including flaviviruses, has two major challenges. The first challenge is the rapid emergence of resistance due to the low fidelity of the viral RNA-dependent RNA polymerase (RdRp). Thus, DAAs against different viral targets should be pursued for combination therapy to mitigate resistance, as exemplified by the optimal therapy of hepatitis C virus. During the past funding period of this CETR consortium (AD3C), we identified potent inhibitors of distinct flavivirus protein functions. In the current proposal, we will develop these inhibitors further into clinical candidates through hit-to-lead optimization. These preclinical candidates will form the foundation of combination therapy. The second challenge is the narrow spectrum of antiviral activity due to the sequence variations of the binding pockets of DAAs. Nucleoside/nucleotide (Nuc) DAAs can overcome this challenge through targeting the most conserved active sites of viral polymerases. This strategy has been used successfully to combat human viral infections, as Nuc drugs possess broad-spectrum activity and a high resistance barrier when treating HIV and HCV patients2. We propose to perform a focused testing of novel Nuc collections from the Emory Institute for Drug Development (EIDD; headed by (b) (3) (A), (b) (6)) and (b) (3) (A), (b) (6) against flaviviruses. In support of this idea, testing of the EIDD Nuc collection against coronaviruses and alphaviruses identified promising inhibitors for Projects 1 and 2 of AD3C. Based on the above rationale, we propose three Specific Aims to develop flavivirus DAAs (see Fig 1).

Aim 1. Develop preclinical DAAs targeting flavivirus capsid and NS4B protein. Previous studies have identified promising inhibitors of DENV capsid (required for flavivirus assembly) and ZIKV NS4B (required for flavivirus replication and immune evasion) proteins. We will perform hit-to-lead optimization to advance these compounds to animal efficacy testing and preclinical development. Specifically, (i) the DENV capsid inhibitor showed in vivo efficacy, but low aqueous solubility. We have crystalized DENV-2 capsid protein in complex with the inhibitor, and will perform structure-based rational design to improve its solubility and efficacy. (ii) The ZIKV NS4B compound exhibited <10 nM EC50 and >25 μM CC50 in cell culture. We will profile the pharmacokinetics (PK) and test the in vivo efficacy of the inhibitor in a ZIKV mouse model. For both compound classes, medicinal chemistry will be performed to broaden the antiviral spectrum to other flaviviruses. In addition, we will passage viruses in the presence of drug candidates to define the potential for resistance, followed by characterization of the resistant viruses for their replication fitness in vitro and pathogenic potential in vivo.

Aim 2. Identify novel Nuc DAAs with pan-flavivirus activity. Using DENV as a pilot, we will identify panflavivirus Nuc inhibitors from the EIDD and (b) (3) (A), (b) compound collections. We will test concurrently (i) a nucleoside (that require host kinases to convert to nucleoside triphosphate for antiviral activity) collection in a cell-based DENV infection assay and (ii) a nucleoside triphosphate collection in a biochemical RdRp assay. Compounds with activities in both assays will be prioritized for development because they are efficiently converted to nucleoside triphosphate by host kinases. Compounds without activity in (i), but with activity in (ii) are not efficiently converted to nucleoside triphosphate by host kinases; we will pursue phosphonate or phosphoramidate prodrugs to rescue these Nucs for development. For identified Nucs, we will characterize their spectrum against other flaviviruses, confirm mechanism of action, assess in vivo protection, and evaluate resistance.

Aim 3. Evaluate combination therapy directed against flavivirus infection. Combination therapy is essential to prevent resistance during antiviral treatment. We will test therapeutic regimens combining DAAs with distinct viral targets from Aims 1 and 2. We will first examine the synergistic or additive effects of DAAs in cell culture. The most effective combinations from cell culture will be evaluated in mouse pathogenesis models. Finally, the most promising DAA combination will be validated in non-human primate (NHP) models of flavivirus infection and will be used to guide future clinical implementation.

We will deliver the proposed milestones by combining the cutting-edge biology from academia and drug discovery acumen from industry along with defined Go/No-Go decision algorithms and with input from the Scientific Advisory Committee (SAC). The collective effort addresses a major unmet medical need by bridging academia and industry, including investigators from University of Texas Medical Branch (Pei-Yong Shi), Washington University in St. Louis (b) (3) (A), (b) (6) Oregon Health Science University (b) (3) (A), (b) (6) EIDD (b) (3) (A), (b) (6) Southern Research (Ashish Pathak), and (b) (3) (A), (b) (6)

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: B2 Yr 1 Project 3 flavi annual report Dec 10 2019-sm.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

Not Applicable

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

File uploaded: B4 Yr 1 Project 3 flavi annual report Dec 10 2019-sm.pdf

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

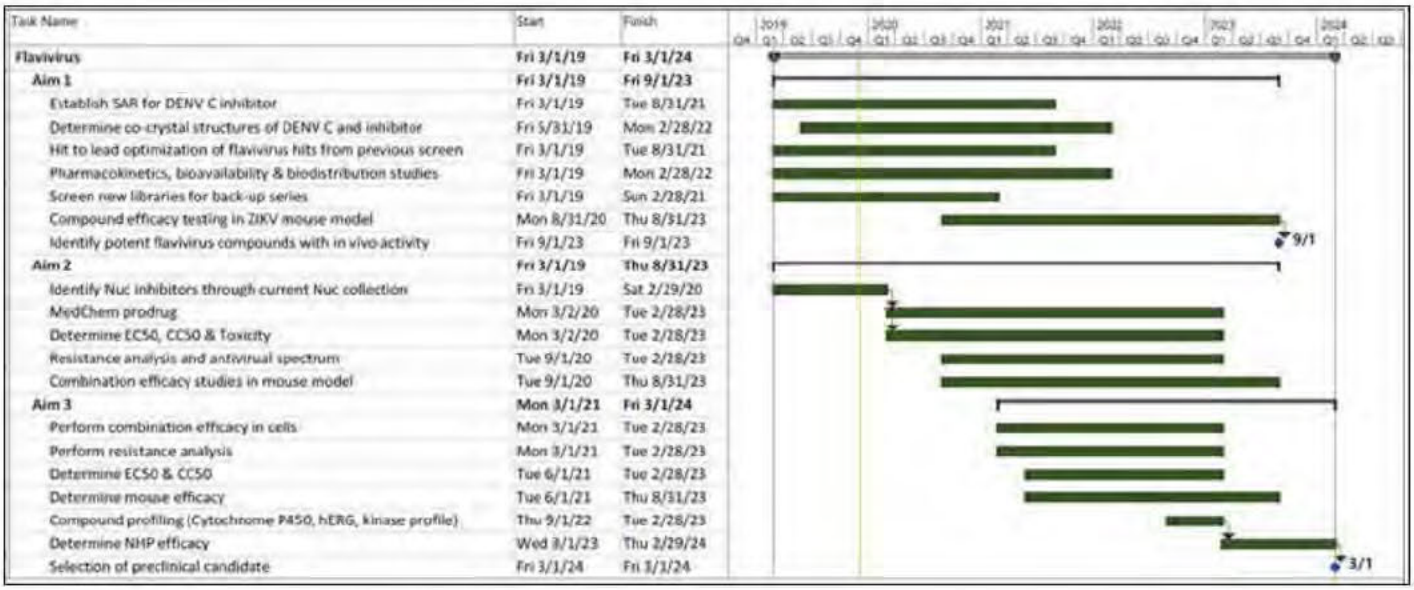
B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

We will pursue the project as proposed in the original grant in the coming year (see the tasks and timeline table in B.2). Briefly, we will continue the following promising directions.
•Perform cocrystal structure-based rational design of dengue capsid inhibitors to achieve improved antiviral spectrum and physicochemical properties.
•Complete hit-to-lead medchem effort on SRI-39748 and SRI-39757 scaffolds (derived from previous screens) to reach go/no-go decision.
•Continue to pursue the Gilead compound for in vivo efficacy in Zika mouse model.
•Test in vivo efficacy for three potential pan-flavivirus nucleoside/nucleotide inhibitors (SRI-43918, EIDD-1931/EIDD-2801, and Remdesivir)
•Complete dengue screen of 200,000 compounds

Project 3 B.2 Accomplished under goals:

Major activities and objectives: We have made significant progress on the flavivirus antiviral project. Inhibitors of Zika virus (ZIKV), dengue virus (DENV), and pan-flavivirus are being pursued in parallel. The new chemical entities are derived from Southern Research, [redacted] (b) (6), (b) (3) (A). The proposed project milestones have been met according to the original timelines.

Task Name	Start	Finish
Flavivirus	**Fri 3/1/19**	**Fri 3/1/24**
Aim 1	**Fri 3/1/19**	**Fri 9/1/23**
Establish SAR for DENV C inhibitor	Fri 3/1/19	Tue 8/31/21
Determine co-crystal structures of DENV C and inhibitor	Fri 5/31/19	Mon 2/28/22
Hit to lead optimization of flavivirus hits from previous screen	Fri 3/1/19	Tue 8/31/21
Pharmacokinetics, bioavailability & biodistribution studies	Fri 3/1/19	Mon 2/28/22
Screen new libraries for back-up series	Fri 3/1/19	Sun 2/28/21
Compound efficacy testing in ZIKV mouse model	Mon 8/31/20	Thu 8/31/23
Identify potent flavivirus compounds with in vivo activity	Fri 9/1/23	Fri 9/1/23
Aim 2	**Fri 3/1/19**	**Thu 8/31/23**
Identify Nuc inhibitors through current Nuc collection	Fri 3/1/19	Sat 2/29/20
MedChem prodrug	Mon 3/2/20	Tue 2/28/23
Determine EC50, CC50 & Toxicity	Mon 3/2/20	Tue 2/28/23
Resistance analysis and antiviral spectrum	Tue 9/1/20	Tue 2/28/23
Combination efficacy studies in mouse model	Tue 9/1/20	Thu 8/31/23
Aim 3	**Mon 3/1/21**	**Fri 3/1/24**
Perform combination efficacy in cells	Mon 3/1/21	Tue 2/28/23
Perform resistance analysis	Mon 3/1/21	Tue 2/28/23
Determine EC50 & CC50	Tue 6/1/21	Tue 2/28/23
Determine mouse efficacy	Tue 6/1/21	Thu 8/31/23
Compound profiling (Cytochrome P450, hERG, kinase profile)	Thu 9/1/22	Tue 2/28/23
Determine NHP efficacy	Wed 3/1/23	Thu 2/29/24
Selection of preclinical candidate	Fri 3/1/24	Fri 3/1/24



B.2.a DENV capsid inhibitor: We have solved the cocrystal structure of dengue virus serotype-2 capsid (DENV-2 C) in complex of inhibitor ST148 (1.5 Å resolution; **Fig. 1**). The cocrystal structure has revealed a novel antiviral mechanism to target viral capsid. The structural information has also uncovered the mechanism of resistance and antiviral spectrum. The cocrystal enables rational design of analogs with improved compound solubility and antiviral spectrum.

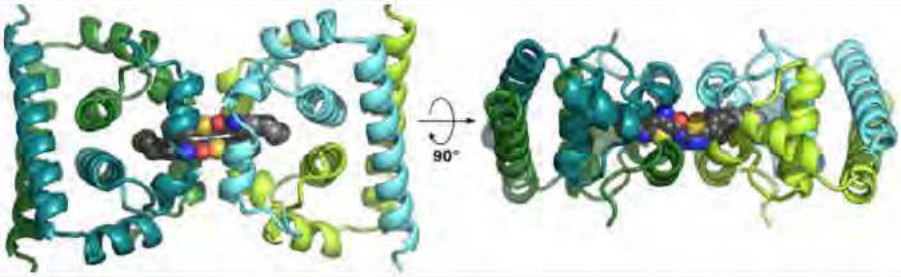
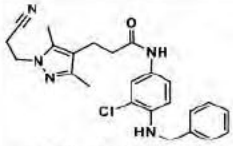


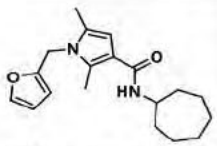
Fig 1. Cocrystal structure of DENV-2 C protein in complex with inhibitor ST148. Structure of DENV-2 C tetramer in complex with ST148. Two C dimers form a "kissing" tetramer. Two ST148 molecules bind to a pocket at the tetramer interface. ST148 and C protein tetramer are presented as CPK and cartoon, respectively.

B.2.b ZIKV inhibitor: Inhibitors identified from previous high-throughput screening have undergone "hit-to-lead" optimizations. Specifically, we have identified two promising scaffolds of ZIKV inhibitors (SRI-39748 & SRI-39757) currently under hit-to-lead optimization. SAR has been established for these scaffolds to minimize the liabilities of these inhibitors.

We have demonstrated in vivo efficacy for one class of ZIKV inhibitor in A129 mouse model **(Fig. 2)**. One inhibitor from Gilead has shown promising mouse efficacy with ~100-fold viremia reduction. In addition, the compound completely prevented weight loss or death of infected mice.



SRI-39748
EC_{90} 0.13 µM
CC_{50} >40 µM
Solubility 7 µM
LogD 3.2
HLM $t_{1/2}$ 6 min
MLM $t_{1/2}$ 1 min



SRI-39757
EC_{90} 4.7 µM
CC_{50} >40 µM
Solubility 23 µM
LogD 3.7
HLM $t_{1/2}$ 9.5 min
MLM $t_{1/2}$ 2.9 min

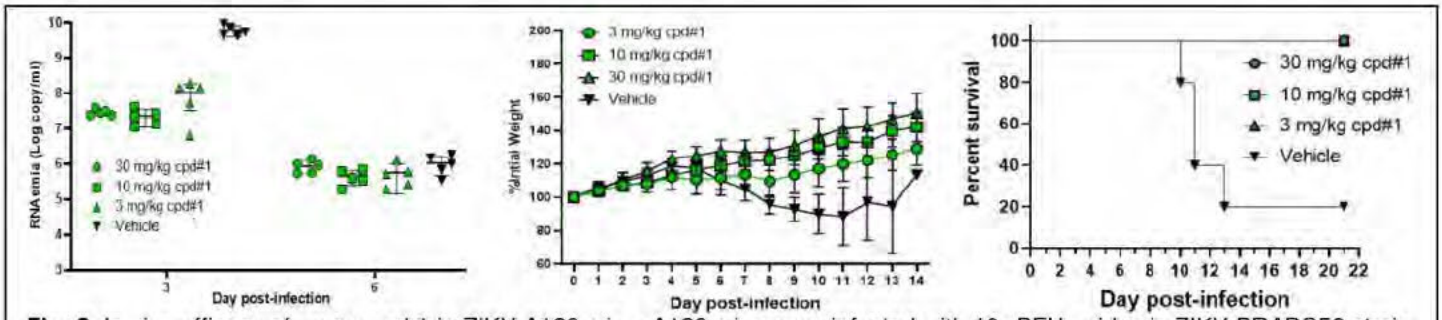
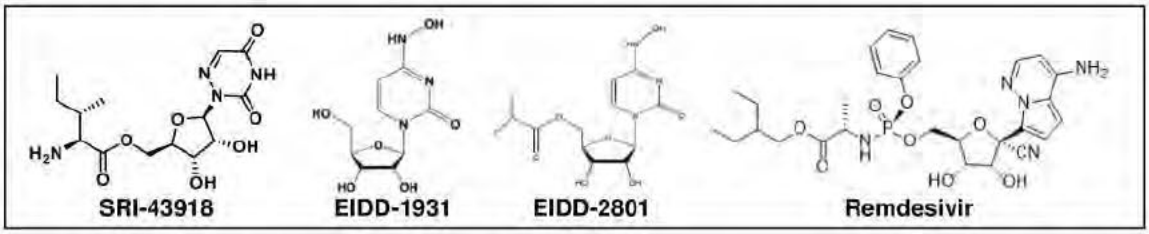


Fig. 2. In vivo efficacy of compound 1 in ZIKV A129 mice. A129 mice were infected with 10_5 PFU epidemic ZIKV PRABC59 strain, immdiately treated daily with indicated dose of compound, and monitered for viremia (left), weight loss (middle), and survival (right).

B.2.c Pan-flavivirus inhibitor: We have taken the following three approaches to identify pan-flavivirus inhibitors. As expected, nucleoside/nucleotide analogs are likely to have pan-flavivirus activities.

- We have identified Southern Research nucleotide inhibitor SRI-43918. SRI-43918 has pan-DENV partitivity with EC_{50} of 1-6 μM and CC50 >50 μM. We are currently verifying the mode-of-action of the compound and planning in vivo efficacy test.



- EIDD-1931 and its ester prodrug EIDD-2801, nucleoside/nucleotide from [redacted] (b) (6), (b) (3) (A) have been identified with anti-flavivirus activities in cell culture. Optimal dose and route will be explored for in vivo activity.
- We have obtained Remdesivir [redacted] (b) (6), (b) (3) (A) and tested its activity against flaviviruses. We found Remdesivir has pan-flavivirus activity in cell culture against DENV-1 to -4, WNV, JEV, ZIKV, and YFV (EC_{50} 0.1-1.0 μM). The cell culture efficacy justifies to test Remdesivir in WNV and ZIKV mouse models.

It should be noted that ester prodrugs are commonly used to improve nucleoside/nucleotide drug absorption and cellular membrane penetration. In humans, intracellular carboxylesterase degrades the ester moiety and releases the nucleoside/nucleotide for triphosphorylation. Because mice express a high level of secreted carboxylesterase 1c (*Ces1c*, which is absent in human plasma), the secreted carboxylesterase 1c in mouse plasma rapidly degrades the ester moiety of the prodrug before the compound enters cells. Therefore, for compounds with an ester prodrug moiety (such as SRI-43918, EIDD-2801, and Remdesivir), we may have to use *Ces1c*$^{-/-}$ mice for efficacy testing. To overcome this challenge, **we have obtained *Ces1c*$^{-/-}$ mice from Gilead**. Besides direct use for efficacy testing, the *Ces1c*$^{-/-}$ mice will be crossed with A129 or AG129 mice to create double knockout mice for DENV and/or ZIKV testing. The double knockout mice will be a critical reagent for the field of antiviral research.

B.2.d Screening effort for new dengue inhibitors. To ensure a robust flavivirus pipeline, we have initiated a new screen of 200,000 compounds. These compounds have not been tested in our previous screen. We will prioritize compounds with pan-DENV or pan-flavivirus activities. In addition, due to the sequence variations among the four serotypes of DENV, we plan to perform a pilot screen using DENV-4. This is because DENV-4 is more phylogenetically distant from DENV-2 that has been the only serotype for dengue drug discovery in the past two decades. We believe a DENV-4 screen may identify hits with different antiviral profiles.

Project 3 B.4 Training opportunities: All students, postdocs, and technicians have had the opportunity to learn real drug discovery through this project. These hands-on experiences are invaluable for their career development and future research selection. Besides performing experiments, they also participate in data analysis, project discussion, manuscript preparation, and presentation at conferences. These trainees are well positioned for potential industry career opportunities.

C. COMPONENT PRODUCTS

C.1 PUBLICATIONS

Not Applicable

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Not Applicable

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Not Applicable

C.5 OTHER PRODUCTS AND RESOURCE SHARING

Nothing to report

D. COMPONENT PARTICIPANTS

Not Applicable

E. COMPONENT IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

Not Applicable

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

NOTHING TO REPORT

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?
Not Applicable

F. COMPONENT CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. COMPONENT SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

Not Applicable

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Not Applicable

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT

Not Applicable

G.10 ESTIMATED UNOBLIGATED BALANCE

Not Applicable

G.11 PROGRAM INCOME

Not Applicable

G.12 F&A COSTS

Not Applicable

RESEARCH & RELATED BUDGET - SECTION A & B FINAL

ORGANIZATIONAL DUNS*: 0685522070000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: Washington University

Start Date*: 03-01-2020 **End Date*:** 02-28-2021

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		(b) (6), (b) (3) (A)				Project Lead	(b) (6), (b) (4)	(b) (6)			9,615.00	2,260.00	11,875.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **11,875.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	(b) (6)			38,071.00	11,138.00	49,209.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
4	1 Lab Assistant/3 Technicians				28,219.00	11,053.00	39,272.00
5	**Total Number Other Personnel**					**Total Other Personnel**	**88,481.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**100,356.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E

ORGANIZATIONAL DUNS*: 0685522070000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: Washington University

Start Date*: 03-01-2020 **End Date*:** 02-28-2021

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	2,499.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**2,499.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		0.00
2. Stipends		0.00
3. Travel		0.00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K

ORGANIZATIONAL DUNS*: 0685522070000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: Washington University

Start Date*: 03-01-2020 **End Date*:** 02-28-2021

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	64,141.00
2. Publication Costs	1,666.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8. Other Expenses	4,998.00
9. Supplies-Animal Purchases only	4,165.00
10. Other Expenses-Animal Expenses only	53,895.00
Total Other Direct Costs	**128,865.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**231,720.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. Federal MTDC	52.5	231,720.00	121,653.00
		Total Indirect Costs	**121,653.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**353,373.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification* File Name: Budget Justification (b) (6), (b) (3) (A) [redacted].pdf

(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

BUDGET JUSTIFICATION

[redacted]

PERSONNEL:

[redacted] **M.D., Ph.D. Principal Investigator.** [redacted] will devote [redacted] **calendar months** to this project and receive [redacted] calendar months of salary support. He will be responsible for the design and oversight of all investigations occurring in [redacted] laboratory. He will actively participate in the planning and execution of experiments, as well as in the generation of progress reports and manuscripts.

[redacted] **i, Ph.D. Research Associate.** [redacted] He has significant experience in evaluating flavivirus pathogenesis and analyzing correlates of protection in vivo. He will devote [redacted] **calendar months** [redacted] effort) of time to this project and derive commensurate salary from it. He will be responsible for performing many of the *in vivo* experiments with the lead compounds and analyzing virological and pathological endpoints. He will also help interpret data and troubleshoot technical problems in consultation with the principal investigator.

Technical Staff. We have a team of three technicians below who will work with [redacted] to perform in vivo therapeutic studies and evaluation. We use a team approach to maximize flexibility given some compounds require multiple dosing throughout the day. All three technicians are trained in in vivo studies with flaviviruses. (a) [redacted], **Technician.** He will devote [redacted] **calendar months** [redacted] effort) of time to this project and derive commensurate salary from it;

(b) [redacted], **Technician.** [redacted] will devote [redacted] **calendar months** [redacted] effort) of time to this project and derive commensurate salary from it;

(c) [redacted], **Technician.** He will devote [redacted] **calendar months** [redacted] effort) of time to this project and derive commensurate salary from it.

[redacted] **Lab Assistant.** He will devote [redacted] **calendar months** [redacted] effort) of time to this project and derive commensurate salary from it. [redacted] is responsible for washing glassware and autoclaving.

SUPPLIES:

Tissue culture ($16,660). With this project, there will be a considerable amount of tissue culture. The funds requested will be used for media preparation, serum, antibiotics, plasticware (disposable pipets, pipetman tips, flasks, tubes, cryogenic vials, filtration flasks, sterile bottles), cytokine supplements, and hybridoma and antibody growth. In particular, defined serum ($300/bottle), growth factor supplements, primary cell cultures, and are expensive to propagate. In addition, we will need to grow large stocks of viruses and titrate tissue homogenates for viral yield.

Molecular Biology Reagents ($10,413). This amount has been budgeted for reagents for molecular cloning (restriction enzymes), proteases, transfection (liposomes, electroporation cuvettes), plasmid DNA and RNA purification kits, mutagenesis, vectors, bacterial culture supplies, and DNA/RNA/protein electrophoresis. This will be especially important for many of the mechanism of action studies with antibodies.In addition, some of these funds will be used for a sensitive and reproducible quantitative real-time RT-PCR for assessing alphavirus replication using an ABI 7700 Sequence Detection instrument. This assay has become our standard for RNA quantitation and will be used to measure viral RNA levels in serum and in tissues. RT-PCR reagents, primers, TaqMan probes cost approximately $1 per well. In addition, for most samples, a ribosomal or actin RNA control is run for normalization purposes.

General ($12,495). This amount has been budgeted for general chemical supplies including buffers, salts, organic solvents, acids, bases, and detergents, as well as radiochemicals and disposal, when needed. We also will purchase oligonucleotides that will be used for cloning and sequencing. Alos, these funds will be used for purchasing small equipment (pipetteman (multi- single- serological), vortexes, waterbaths, microcentrifuges) and disposable tubes and tips. Finally, this amount includes funds for liquid N2/CO2 bottled gas and related tank rental fees, and glasswashing,

MAR1-5A3 anti-Ifnar1 antibodies ($5,831). To generate lethal challenge models of ZIKV mice are administered a 2 mg dose of MAR1-5A3. This will be used in many of our protection models. MAR1-5A3 will be purchased commercially. We expect to used ~1 g of mAb per year for the challenge studies, which can be purchased from (b) (6), (b) (3) (A)

Immunochemical Reagents ($12,495). This has been budgeted for the direct labeling of multiple antibodies with different fluorophores, for secondary reagents for ELISA, and for intracellular immunofluorescence studies. We also will use part of these funds for analysis of purified MAbs for *in vivo* studies. Finally, this amount is for reagents associated with running of the flow cytometer including calibration, buffers, controls, and software licenses. Some of our high-throughput analyis will utilize flow-cytometry based readouts. In addition, we will use this equipment for analyzing viral infection in different tissues.

Animal purchase or cost ($29,155). This cost reflects the purchase of WT C57BL/6 mice or the cost of producing human STAT2-KI mice from our own breeding colony. The latter is more expensive given their smaller litter sizes. In addition, some of the studies will be performed in pregnant animals, which is more costly to set up.

OTHER EXPENSES:

Animal Breeding ($6,664). This amount is needed to maintain breeders of the strains used in this project to generate enough progeny to perform large scale, controlled experiments.

Animal housing (18,076). This cost reflects the cage charges for all mice immediately before and after infection with ZIKV. All infection experiments will take place in the ABSL3, which has higher per diems. A detailed estimate of the number of mice to be used is provided in the Vertebrate Animals section.

Pharmacy, Surveillance, and Dissection tools ($4,165). This is required for sedation, euthanasia, necropsy, and surveillance for adventitious pathogens within the animal facility

BSL costs ($6,248). This includes additional costs associated with BSL2 and A-BSL3 biosafety laboratory operation (facilities access charges and user fees, personal protective equipment, and BSL3 waste disposal). We have enhanced the safety level of animal studies to A-BSL3 due to added risk of handling infected animals.

Equipment Service Contracts ($4,998). Funds are budgeted for service contracts for laboratory equipment (ELISPOT reader, Miltenyi 8-color flow cytometer, TaqMan ABI 7800, ultracentrifuge).

Other Costs:

Funds are budgeted for publication costs ($1,666) and travel three times per year to a special progress meeting with the members of the CETR ($2,499) as well; as other scientific and Project specific meetings.

NOTE: The numbers in this justification reflect a mandated 16.7% reduction on all projects

A. COMPONENT COVER PAGE

Project Title: Project 4.2: Identify New Therapies Targeting Influenza Replication

Component Project Lead Information:

TEKWANI, BABU L

B. COMPONENT ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

SPECIFIC AIMS
Specific aims: The overall goal of this project is to identify new therapies that target influenza virus replication. The global health burden of annual influenza infections and periodic epidemics coupled with the emergence of avian influenza viruses (AIVs), like H5NX and H7N9, highlight the urgent need for new effective treatments. A primary concern with the current influenza therapies (M2 and neuraminidase inhibitors [NAIs]) is the development of resistance mutations that can negate therapeutic benefit. Both published evidence and clinical experience suggest strongly that targeting the influenza virus RNA dependent RNA polymerase (RdRp) complex is a rational approach for antiviral therapy. This complex is responsible for many viral functions, including 5´ cap recognition, endonucleolytic cleavage, RNA synthesis, and polyadenylation. Clearly, the trimeric RdRp complex provides multiple functional domains as targets for antiviral drug therapy and combination therapy. Agents can target the distinct functions and, theoretically, reduce the minimize development of resistance since resistance mutations would likely reduce the fidelity of the RdRp. One oral agent targeting the endonuclease domain, baloxavir, has shown efficacy in uncomplicated influenza and was recently approved in Japan for treating influenza in adults and children. A second oral agent targeting the 5´ cap binding domain, pimodivir, has shown antiviral activity in uncomplicated influenza and is advancing in clinical development. A third oral agent, favipiravir, targeting the polymerase activity has been approved in Japan for treating novel influenza strains not inhibited by NAIs.

We have recently identified several potent molecules from the Emory Institute for Drug Discovery (EIDD), (b) (6), (b) (3) (A), and Southern Research (SR). This application and research team provides the medicinal chemistry expertise, follow up assays, and In vivo experience to transform active hits into lead compounds and advance them rapidly into animal models of influenza virus infection. The public private partnership with EIDD and GS leverages the proposed developmental program and promises to yield new classes of highly active
molecules that target the RdRp complex. We propose the following Specific Aims:

Aim 1. Identify potent influenza compounds targeting RdRp complex functions
A. Continue hit to lead optimization of the active compounds from EIDD, GS, and SR.

Hypothesis and rationale: We hypothesize that hits identified from ongoing studies that target the RdRp complex can serve as the basis for a lead optimization program. This includes novel chemical classes from EIDD, SR, and GS. We will use an iterative medicinal chemistry effort that addresses potency (antiviral activity) as part of the Medicinal Chemistry and Lead Development Core (MCLDC). The focus will be on the essential activities of this complex, which provides fertile ground for drug discovery due to the number of conserved functional domains, and interactions that could be targeted. Our goal is to identify compounds directed against the conserved functions of the RdRp that are active against multiple subtypes of influenza virus.

Approach: The initial hits from EIDD, GS, and the SR compound collection will undergo hit to lead chemistry. Additional analogs will be evaluated and related compounds synthesized. Compounds with effective concentrations below 5 µM will be evaluated further to assess their activity against multiple subtypes of the virus.

Aim 2: Define the spectrum of antiviral activity and define mechanism of action of the lead compounds
A. Define spectrum of antiviral activity against wild-type, AIV, and NAI resistant strains of influenza virus
B. Characterize the mechanism of action (MOA) against RdRp functions
C. Map molecular determinants of resistance and their impact on susceptibility and replication fitness
D. Perform combination studies and evaluate lead molecules from other three Projects

Hypothesis and rationale: Active molecules presented here will be evaluated to confirm their activity against multiple subtypes of the virus including AIVs and preliminary data already have identified compounds that appear to inhibit at least three distinct RdRp functions. Additional assays in an appropriate setting of primary human small airway epithelial cells and biochemical assays will identify domains targeted by the most active compounds.

Experimental strategy: Several secondary and tertiary assays are in place to identify rapidly compounds that specifically inhibit the functions of the RdRp complex. The lead compounds also will be used to generate resistant isolates so that the molecular determinants of resistance can be mapped and confirmed in engineered viruses.

Aim 3: In vivo evaluation and bioavailability studies.
A. Perform maximum tolerated dose and pharmacokinetic studies in mice
B. Evaluate efficacy against mouse-adapted A/California/04/2009 (H1N1) infection in mice
C. Characterize efficacy of lead compound in ferrets infected with A/California/04/2009 (H1N1)

Hypothesis and rationale: Pharmacokinetic, bioavailability, distribution, and clearance studies will prioritize the best chemical scaffolds for further development and help inform the design of studies in mice and ferrets for pivotal efficacy experiments In vivo.

Experimental strategy: Compounds with the best efficacy from Aims 1 and 2 will be prioritized to identify molecules with drug-like properties for efficacy studies in murine and ferret models of influenza virus infection.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: B.2 AD3C Project 4-Y1report.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

Not Applicable

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

NOTHING TO REPORT

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

•Initiate hit-to-lead studies on 1-2 HTS hits.
•Synthesis and evaluation of additional Pimodivir analogs in RdRp, CPE and VLR assays.
•Continue SAR on nucleoside SRI-7958 to improve anti-flu activity.
•Screen compounds (all projects) for DHODH inhibition.
•Establish RdRp cell free assay.
•New RdRp target: ANP32A-PB2 interactions for anti-flu drug discovery
•Perform NMR docking to map active sites
•Characterize mechanism of action of lead compounds: RpRd functions
•Combination Studies: Efficacy of best lead compounds will be evaluated in combination with oseltamivir as well as with baloxavir, pimodivir, and favipiravir.
•In vivo evaluation of 1-2 lead compounds in mouse models of influenza

B.2 Project 4: Identification and Characterization of Novel Drugs that Target the Influenza Virus Polymerase Functions

General Progress summary

- HTS Screening of 200,000 compounds (ChemDiv collection) using a Mirrorball immunofluorescent assay to measure M2, identified 140 confirmed hits with IC_{50} < 30μM and CC_{50} > 40μM.
- 31 compounds from HTS screening were selected for follow-up evaluation after clustering analysis, pain filtration and visual inspections of the structures of the compounds.
- 28 compounds were procured; 26 compounds passed QC tests and screened against H3N2 and H1N1.
- 11 compounds were active against H3N2 and only one compound was also active against H1N1. None of the 11 compounds from HTS were active against Dihydroorotate Dehydrogenase (DHODH).
- SRI-44249 (active against both H1N1 & H3N2) selected for re-synthesis and follow-up
- SRI-44218, SRI-44251, SRI-44209, SRI-44213 also selected for potential follow-up studies
- 18 Nucleoside analogs screened against H3N2; 5 analogs active, no DHODH inhibition.
- DHODH counter screen assay established with recombinant hDHODH enzyme.
- Recombinant human DHODH was produced at Southern Research.
- Mini genome and Cap snatching RdRp assays were standardized.

Antiviral Assays

- Mirrorball® immunofluorescence assay detecting surface expression of M2 protein (H3N2 & H1N1/A549) (HTS and SAR) (Southern Research)
- CPE Assay (H3N2 & H1N1/MDCK) (SAR and Follow-up) (Southern Research)
- Virus Load Reduction assay (H1N1/A549-MDCK) (Follow-up) (UAB)

Mechanism of Action/Molecular Target Assays

- RdRp Cap snatching assay (UAB)
- Mini-genome cell based transcription assay (Southern Research)
- Human Dihydroorotate Dehydrogenase (DHODH) inhibition assay was setup as a counter screen to exclude the DHODH inhibitors from further follow-up. (Southern Research)

SAR Driving Assays

- 131 novel compounds tested (March-October, 2019) at ten concentrations
- Mirrorball IFA used to determine IC_{50} values against H3N2 (Udorn) and H1N1 (Wisconsin)
- Evaluation of cytotoxic effect on A549 cells at 72 hours; used to determine CC_{50} values

HTS Campaign

- Paucity of hits from previous screens using the ELVIRA luciferase reporter assay and a CPE assay (screened Enamine and ChemBridge compound libraries)

- Additional chemical matter needed for the Influenza project
- Screened another 200,000 compounds (ChemDiv collection) using a Mirrorball immunofluorescent assay to measure M2 protein levels in H3N2 infected A549 cells (same assay used to drive SAR)
- Identified 140 confirmed hits with IC_{50} < 30μM and CC_{50} > 40μM
- 10 VX787 analogs tested in RdRp assays and also against H3N2; one analog (SRI-43679) active. SRI-43679 was also active against H1N1 and H3N2. None of the 10 VX787 analogs were active against DHODH.
- There were 125 compounds tested against DHODH counter-screen; 23 active compounds were tested for dose-response to determine relative selectivity (antiviral activity vs DHODH inhibition).
- EIDD-2838 is a broadly active ribonucleotide-5'-monophosphate analog with activity against influenza A and B strains (EC_{50} ~1μM) in CPE models of infection. EIDD-2838 was found to be an influenza cap-snatching inhibitor (EC_{50} = 2-4 μM) through the AD3C.

C. COMPONENT PRODUCTS

C.1 PUBLICATIONS

Not Applicable

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Not Applicable

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Not Applicable

C.5 OTHER PRODUCTS AND RESOURCE SHARING

Nothing to report

D. COMPONENT PARTICIPANTS

Not Applicable

E. COMPONENT IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

Not Applicable

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

NOTHING TO REPORT

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?
Not Applicable

F. COMPONENT CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. COMPONENT SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

Not Applicable

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Not Applicable

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT

Not Applicable

G.10 ESTIMATED UNOBLIGATED BALANCE

Not Applicable

G.11 PROGRAM INCOME

Not Applicable

G.12 F&A COSTS

Not Applicable

RESEARCH & RELATED BUDGET - SECTION A & B FINAL

ORGANIZATIONAL DUNS*: 0069005260000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: Southern Research Institute

Start Date*: 03-01-2020 **End Date*:** 02-28-2021

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	[redacted] (b) (6), (b) (3) (A)				Project Lead	0.00	(b) (6)			17,104.00	7,303.00	24,407.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **24,407.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	(b) (6)			54,943.00	23,461.00	78,404.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
12	1 Biologist/7 Drug Dvlpmt Tech/4 Drug Dvlpmt Suppt				64,211.00	27,418.00	91,629.00
13	**Total Number Other Personnel**					**Total Other Personnel**	**170,033.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**194,440.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E

ORGANIZATIONAL DUNS*: 0069005260000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: Southern Research Institute

Start Date*: 03-01-2020 **End Date*:** 02-28-2021

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	5,238.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**5,238.00**

E. Participant/Trainee Support Costs		Funds Requested ($)*
1. Tuition/Fees/Health Insurance		0.00
2. Stipends		0.00
3. Travel		0.00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K

ORGANIZATIONAL DUNS*: 0069005260000
Budget Type*: ● Project ○ Subaward/Consortium
Enter name of Organization: Southern Research Institute

Start Date*: 03-01-2020 **End Date*:** 02-28-2021

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	111,283.00
2. Publication Costs	0.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8. Other Charges	6,511.00
Total Other Direct Costs	**117,794.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**317,472.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. OH - Salaraies + Benefits	120.0	194,440.00	233,328.00
2. G&A - Salaries + Benefits + OH + ODC	20.0	550,800.00	110,160.00
3. FCCM OH	5.8	194,440.00	11,278.00
4. FCCM G & A (Actual rate is 0.10%)	1.0	550,800.00	551.00
		Total Indirect Costs	**355,317.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS / Daryl W. Mayes / 301-492-4855

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**672,789.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification* File Name: Project 4 Budget Justification Yr 2 Final.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Key Personnel - Biochemistry and Molecular Biology

[redacted] ***Ph.D.*** *(Y2: [redacted] cal. months)*, Distinguished Fellow and Chair Infectious Diseases will serve as Project Lead for Project 4. [redacted] has more than [redacted] years' experience in the areas related to new anti-infective & anti-parasitic drug discovery, bioassays, pharmacology, toxicology, animal models and molecular drug targets. He will supervise and perform available secondary confirmatory assays on hit compounds found in the primary HT screening against IAV. He will also design, and supervise tertiary assays for mechanistic characterization. In particular, *in vitro* assays to discern compound activity on polymerization, endonuclease activity, cap-binding or subunit interaction of the RdRp complex. He will assist in the designing of experiments to these aims and presentation of data and manuscripts, as well as participate in Project meetings.

Other Personnel- Biochemistry and Molecular Biology

[redacted] ***Ph.D.*** *(Y2: [redacted] cal. months).* [redacted] is a Scientist I in [redacted] laboratory, who will conduct secondary screening of hit compounds against an influenza virus available *in vitro* polymerase assay (Aim 2). Additionally, [redacted] will perform experiments to optimize and implement tertiary *in vitro* endonuclease and polymerase assays (Aim 2) to determine the mechanism of action of hit compounds on the RdRp complex (Aim 2). He will be supervised by [redacted]

[redacted] ***M.S.*** *(Y2: [redacted] cal. months).* [redacted] is a Scientist II in the Drug Discovery Division. [redacted] will be responsible for growing and determining the titer of influenza virus stocks to make them available in downstream confirmatory assays and mode of action studies (Aim 1 & 2). [redacted] will also conduct secondary screening of hit compounds against different influenza virus subtypes including plaque reduction, cytopathic effect (CPE) hemaglutination, and neuramindase assays. [redacted] will also assist with BSL-3 evaluations of antiviral compounds for broad efficacy (Aim 2). [redacted] will be supervised by [redacted] for secondary mechanistic assays.

Other Personnel – Animal Influenza Studies

[redacted] ***Ph.D.*** *(Y2: [redacted] cal. months).* [redacted] is a multi-disciplinary research scientist with over [redacted] years of experience in bacteriology, virology, immunology and molecular biology, and is experienced in designing plans for validating or qualifying bioanalytical and immunological assays for analyzing non-clinical and clinical samples. [redacted] responsible for overseeing the influenza virology program, both *in vitro* and *in vivo*, at SR [redacted] designs and executes validations or qualifications and manages sample analysis in partnership with both commercial and government clients. Prior to joining SR in 2015, [redacted] In addition, he supervised and performed a broad range of procedures using novel and standard techniques and methods including refining a number of viral and bacterial animal models. Viral animal models were primarily mouse and ferret influenza models using seasonal, pandemic and highly pathogenic avian influenza strains. He will be responsible for the overall conduct of a multidisciplinary team effort to complete the proposed *in vivo* influenza studies.

(b) (6), (b) (3) (A) *(Y2: 0.08 cal. months)*. (b) (6), (b) (3) (A), Project Manager, DDV, will assist (b) (6), (b) (3) (A) in program management function, including budgets, resource management, and reporting to ensure that the program's deliverables are provided on time and on budget.

In Vivo technical personnel: SR's fully-qualified Operations team, a staff of animal care and equipment technicians, will attend to all study requirements associated with the *in vivo* influenza studies, providing routine animal husbandry and support.

- (b) (6), (b) (3) (A) *(Y2: (b) (6) cal. months)*, Manager of Laboratory Operations, DDV
- (b) (6), (b) (3) (A) *(Y2: (b) (6) cal. months)*, Senior Manager of Vet Services
- (b) (6), (b) (3) (A) *(Y2: (b) (6) cal. months)*, *In Vivo* Technician
- (b) (6), (b) (3) (A), *(Y2: (b) (6) cal. months)*, *In Vivo* Technician
- (b) (6), (b) (3) (A) *(Y2: (b) (6) cal. months)*, Supervisor of Pathology
- (b) (6), (b) (3) (A) *(Y2: (b) (6) cal. months)*, Veterinary Technician
- (b) (6), (b) (3) (A) *(Y2: (b) (6) cal. months)*, Dose Formulation
- (b) (6), (b) (3) (A) *(Y2: (b) (6) cal. months)*, Prosector
- (b) (6), (b) (3) (A) *(Y2: (b) (6) cal. months)*, Sample Prep

The teams of professionals at Southern Research as outlined above are identified as a competent and experienced pool of individuals. We have identified these individuals due to their capabilities, but understand staff changes do occur. Given the timeframe of this Task Order, Southern Research commits to resources of these same capabilities or greater should staffing changes occur.

Other (Non-Labor) Direct Costs *(Y2: $123,032)*

Materials and Supplies *(Y2: $111,283)*. These include labor and materials for cell culture, biochemical assay performance, and general supply costs. These estimates are based upon catalog pricing from commercial vendors (b) (6), (b) (3) (A), historical patterns of laboratory usage, and consumables we already have available from Southern Research. Consumables will include chemicals like buffers, acids and bases, and molecular reagents such as antibodies and flow cytometry reagents. Disposables will include flasks, pipette tips, tissue culture plates, and other miscellanea consumables which are fundamental to the proposed studies.

Animal Costs/In vivo Studies *(Y2: $6,511)*. These costs cover animals, animal care per diems, dosing supplies, bioanalytical supplies, necropsy materials, and all other supporting materials required for Influenza Animal Studies.

Travel *(Y2: $5,238)*. Funds are included for domestic travel by the PI and technical staff to attend the large Center annual meeting for investigators and scientific advisors for two days and also present the results at an appropriate research conferences on antivirals.

A. COMPONENT COVER PAGE

Project Title: Project 3.2: Develop Antiviral Agents for Flavivirus Therapy

Component Project Lead Information:

(b) (6), (b) (3) (A)

B. COMPONENT ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

SPECIFIC AIMS. The flavivirus genus comprises over 70 positive, single-stranded RNA viruses. Many flaviviruses are significant human pathogens, among which dengue virus (DENV) is the most important mosquito-transmitted virus with over 390 million infections per year. West Nile virus (WNV), yellow fever virus (YFV), Japanese encephalitis virus (JEV), tick-borne encephalitis virus (TBEV), and recently Zika virus (ZIKV) also cause global outbreaks and epidemics, posing constant threats to public health1. No FDA-approved antiviral drug is available to treat any flavivirus infection, and vaccines are lacking against several family members. Thus, the development of flavivirus antiviral therapy is a public health priority.

The goal of this proposal is to develop direct antiviral agents (DAAs) for flavivirus therapy. Antiviral development for all RNA viruses, including flaviviruses, has two major challenges. The first challenge is the rapid emergence of resistance due to the low fidelity of the viral RNA-dependent RNA polymerase (RdRp). Thus, DAAs against different viral targets should be pursued for combination therapy to mitigate resistance, as exemplified by the optimal therapy of hepatitis C virus. During the past funding period of this CETR consortium (AD3C), we identified potent inhibitors of distinct flavivirus protein functions. In the current proposal, we will develop these inhibitors further into clinical candidates through hit-to-lead optimization. These preclinical candidates will form the foundation of combination therapy. The second challenge is the narrow spectrum of antiviral activity due to the sequence variations of the binding pockets of DAAs. Nucleoside/nucleotide (Nuc) DAAs can overcome this challenge through targeting the most conserved active sites of viral polymerases. This strategy has been used successfully to combat human viral infections, as Nuc drugs possess broad-spectrum activity and a high resistance barrier when treating HIV and HCV patients2. We propose to perform a focused testing of novel Nuc collections from the Emory Institute for Drug Development (EIDD; headed by (b) (3) (A), (b) (6)) and (b) (3) (A), (b) (6) against flaviviruses. In support of this idea, testing of the EIDD Nuc collection against coronaviruses and alphaviruses identified promising inhibitors for Projects 1 and 2 of AD3C. Based on the above rationale, we propose three Specific Aims to develop flavivirus DAAs (see Fig 1).

Aim 1. Develop preclinical DAAs targeting flavivirus capsid and NS4B protein. Previous studies have identified promising inhibitors of DENV capsid (required for flavivirus assembly) and ZIKV NS4B (required for flavivirus replication and immune evasion) proteins. We will perform hit-to-lead optimization to advance these compounds to animal efficacy testing and preclinical development. Specifically, (i) the DENV capsid inhibitor showed in vivo efficacy, but low aqueous solubility. We have crystalized DENV-2 capsid protein in complex with the inhibitor, and will perform structure-based rational design to improve its solubility and efficacy. (ii) The ZIKV NS4B compound exhibited <10 nM EC50 and >25 μM CC50 in cell culture. We will profile the pharmacokinetics (PK) and test the in vivo efficacy of the inhibitor in a ZIKV mouse model. For both compound classes, medicinal chemistry will be performed to broaden the antiviral spectrum to other flaviviruses. In addition, we will passage viruses in the presence of drug candidates to define the potential for resistance, followed by characterization of the resistant viruses for their replication fitness in vitro and pathogenic potential in vivo.

Aim 2. Identify novel Nuc DAAs with pan-flavivirus activity. Using DENV as a pilot, we will identify panflavivirus Nuc inhibitors from the EIDD and (b) (3) (A), (b) compound collections. We will test concurrently (i) a nucleoside (that require host kinases to convert to nucleoside triphosphate for antiviral activity) collection in a cell-based DENV infection assay and (ii) a nucleoside triphosphate collection in a biochemical RdRp assay. Compounds with activities in both assays will be prioritized for development because they are efficiently converted to nucleoside triphosphate by host kinases. Compounds without activity in (i), but with activity in (ii) are not efficiently converted to nucleoside triphosphate by host kinases; we will pursue phosphonate or phosphoramidate prodrugs to rescue these Nucs for development. For identified Nucs, we will characterize their spectrum against other flaviviruses, confirm mechanism of action, assess in vivo protection, and evaluate resistance.

Aim 3. Evaluate combination therapy directed against flavivirus infection. Combination therapy is essential to prevent resistance during antiviral treatment. We will test therapeutic regimens combining DAAs with distinct viral targets from Aims 1 and 2. We will first examine the synergistic or additive effects of DAAs in cell culture. The most effective combinations from cell culture will be evaluated in mouse pathogenesis models. Finally, the most promising DAA combination will be validated in non-human primate (NHP) models of flavivirus infection and will be used to guide future clinical implementation.

We will deliver the proposed milestones by combining the cutting-edge biology from academia and drug discovery acumen from industry along with defined Go/No-Go decision algorithms and with input from the Scientific Advisory Committee (SAC). The collective effort addresses a major unmet medical need by bridging academia and industry, including investigators from University of Texas Medical Branch (Pei-Yong Shi), Washington University in St. Louis (b) (3) (A), (b) (6), Oregon Health Science University (b) (3) (A), (b) (6), EIDD (b) (3) (A), (b) (6) Southern Research (Ashish Pathak), and (b) (3) (A), (b) (6)

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: B2 Yr 1 Project 3 flavi annual report Dec 10 2019-sm.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

Not Applicable

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

File uploaded: B4 Yr 1 Project 3 flavi annual report Dec 10 2019-sm.pdf

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

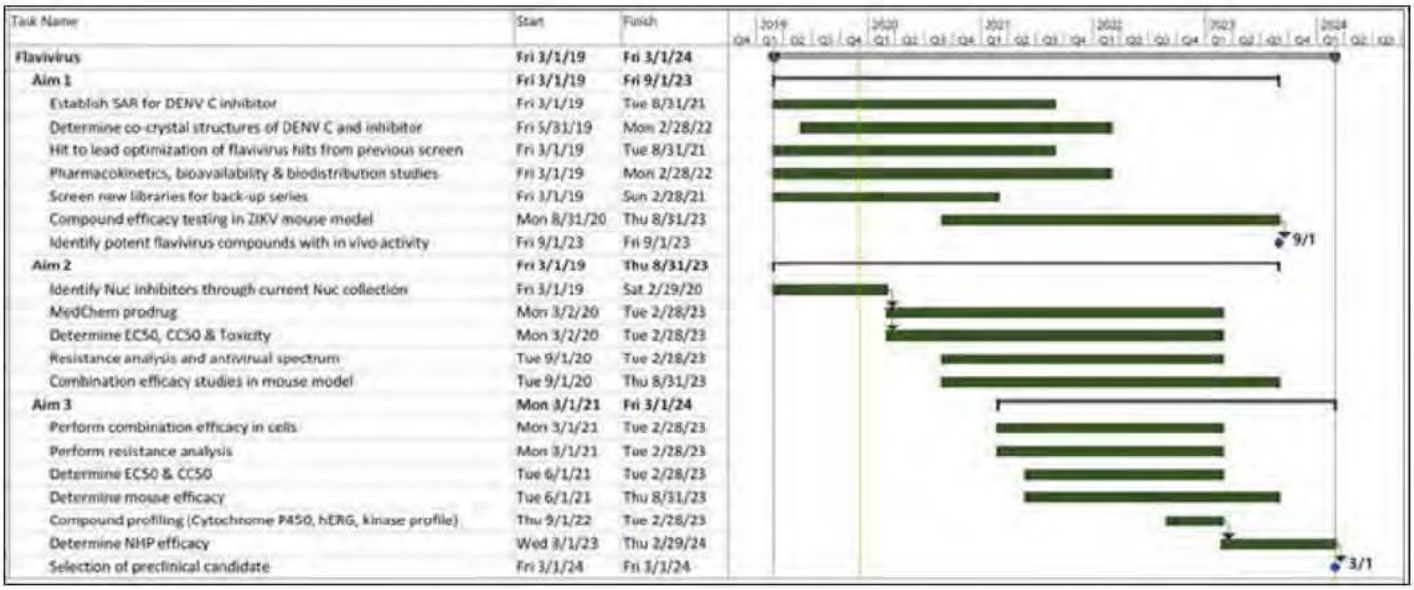
NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

We will pursue the project as proposed in the original grant in the coming year (see the tasks and timeline table in B.2). Briefly, we will continue the following promising directions.
•Perform cocrystal structure-based rational design of dengue capsid inhibitors to achieve improved antiviral spectrum and physicochemical properties.
•Complete hit-to-lead medchem effort on SRI-39748 and SRI-39757 scaffolds (derived from previous screens) to reach go/no-go decision.
•Continue to pursue the Gilead compound for in vivo efficacy in Zika mouse model.
•Test in vivo efficacy for three potential pan-flavivirus nucleoside/nucleotide inhibitors (SRI-43918, EIDD-1931/EIDD-2801, and Remdesivir)
•Complete dengue screen of 200,000 compounds

Project 3 B.2 Accomplished under goals:

Major activities and objectives: We have made significant progress on the flavivirus antiviral project. Inhibitors of Zika virus (ZIKV), dengue virus (DENV), and pan-flavivirus are being pursued in parallel. The new chemical entities are derived from Southern Research, (b) (6), (b) (3) (A). The proposed project milestones have been met according to the original timelines.



Task Name	Start	Finish
Flavivirus	Fri 3/1/19	Fri 3/1/24
Aim 1	Fri 3/1/19	Fri 9/1/23
Establish SAR for DENV C inhibitor	Fri 3/1/19	Tue 8/31/21
Determine co-crystal structures of DENV C and inhibitor	Fri 5/31/19	Mon 2/28/22
Hit to lead optimization of flavivirus hits from previous screen	Fri 3/1/19	Tue 8/31/21
Pharmacokinetics, bioavailability & biodistribution studies	Fri 3/1/19	Mon 2/28/22
Screen new libraries for back-up series	Fri 3/1/19	Sun 2/28/21
Compound efficacy testing in ZIKV mouse model	Mon 8/31/20	Thu 8/31/23
Identify potent flavivirus compounds with in vivo activity	Fri 9/1/23	Fri 9/1/23
Aim 2	Fri 3/1/19	Thu 8/31/23
Identify Nuc inhibitors through current Nuc collection	Fri 3/1/19	Sat 2/29/20
MedChem prodrug	Mon 3/2/20	Tue 2/28/23
Determine EC50, CC50 & Toxicity	Mon 3/2/20	Tue 2/28/23
Resistance analysis and antiviral spectrum	Tue 9/1/20	Tue 2/28/23
Combination efficacy studies in mouse model	Tue 9/1/20	Thu 8/31/23
Aim 3	Mon 3/1/21	Fri 3/1/24
Perform combination efficacy in cells	Mon 3/1/21	Tue 2/28/23
Perform resistance analysis	Mon 3/1/21	Tue 2/28/23
Determine EC50 & CC50	Tue 6/1/21	Tue 2/28/23
Determine mouse efficacy	Tue 6/1/21	Thu 8/31/23
Compound profiling (Cytochrome P450, hERG, kinase profile)	Thu 9/1/22	Tue 2/28/23
Determine NHP efficacy	Wed 3/1/23	Thu 2/29/24
Selection of preclinical candidate	Fri 3/1/24	Fri 3/1/24

B.2.a DENV capsid inhibitor: We have solved the cocrystal structure of dengue virus serotype-2 capsid (DENV-2 C) in complex of inhibitor ST148 (1.5 Å resolution; **Fig. 1**). The cocrystal structure has revealed a novel antiviral mechanism to target viral capsid. The structural information has also uncovered the mechanism of resistance and antiviral spectrum. The cocrystal enables rational design of analogs with improved compound solubility and antiviral spectrum.

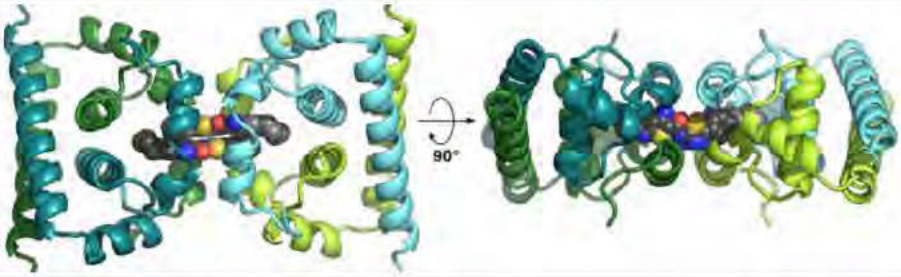
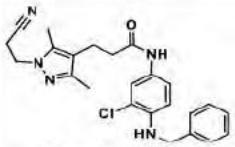
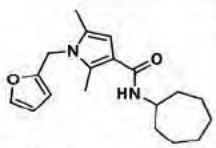


Fig 1. Cocrystal structure of DENV-2 C protein in complex with inhibitor ST148. Structure of DENV-2 C tetramer in complex with ST148. Two C dimers form a "kissing" tetramer. Two ST148 molecules bind to a pocket at the tetramer interface. ST148 and C protein tetramer are presented as CPK and cartoon, respectively.

B.2.b ZIKV inhibitor: Inhibitors identified from previous high-throughput screening have undergone "hit-to-lead" optimizations. Specifically, we have identified two promising scaffolds of ZIKV inhibitors (SRI-39748 & SRI-39757) currently under hit-to-lead optimization. SAR has been established for these scaffolds to minimize the liabilities of these inhibitors.



SRI-39748
EC_{90} 0.13 µM
CC_{50} >40 µM
Solubility 7 µM
LogD 3.2
HLM $t_{1/2}$ 6 min
MLM $t_{1/2}$ 1 min



SRI-39757
EC_{90} 4.7 µM
CC_{50} >40 µM
Solubility 23 µM
LogD 3.7
HLM $t_{1/2}$ 9.5 min
MLM $t_{1/2}$ 2.9 min

We have demonstrated in vivo efficacy for one class of ZIKV inhibitor in A129 mouse model **(Fig. 2)**. One inhibitor (b) (3) (A), (b) (6) has shown promising mouse efficacy with ~100-fold viremia reduction. In addition, the compound completely prevented weight loss or death of infected mice.

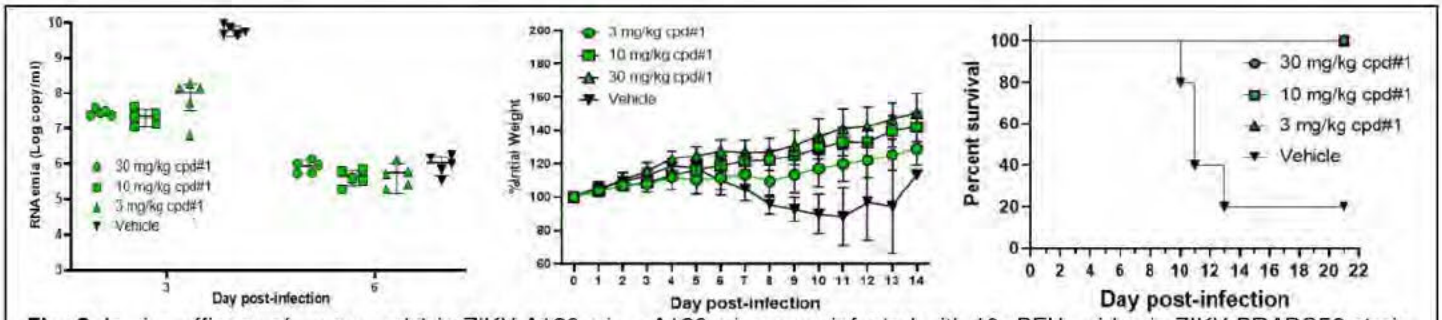
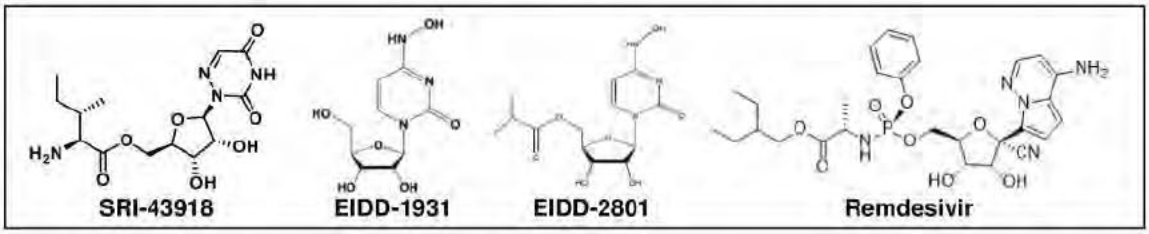


Fig. 2. In vivo efficacy of compound 1 in ZIKV A129 mice. A129 mice were infected with 10_5 PFU epidemic ZIKV PRABC59 strain, immdiately treated daily with indicated dose of compound, and monitered for viremia (left), weight loss (middle), and survival (right).

B.2.c Pan-flavivirus inhibitor: We have taken the following three approaches to identify pan-flavivirus inhibitors. As expected, nucleoside/nucleotide analogs are likely to have pan-flavivirus activities.

- We have identified Southern Research nucleotide inhibitor SRI-43918. SRI-43918 has pan-DENV partitivity with EC_{50} of 1-6 μM and CC50 >50 μM. We are currently verifying the mode-of-action of the compound and planning in vivo efficacy test.



- EIDD-1931 and its ester prodrug EIDD-2801, nucleoside/nucleotide from [redacted] (b) (6), (b) (3) (A) have been identified with anti-flavivirus activities in cell culture. Optimal dose and route will be explored for in vivo activity.
- We have obtained Remdesivir from Gilead and tested its activity against flaviviruses. We found Remdesivir has pan-flavivirus activity in cell culture against DENV-1 to -4, WNV, JEV, ZIKV, and YFV (EC_{50} 0.1-1.0 μM). The cell culture efficacy justifies to test Remdesivir in WNV and ZIKV mouse models.

It should be noted that ester prodrugs are commonly used to improve nucleoside/nucleotide drug absorption and cellular membrane penetration. In humans, intracellular carboxylesterase degrades the ester moiety and releases the nucleoside/nucleotide for triphosphorylation. Because mice express a high level of secreted carboxylesterase 1c (*Ces1c*, which is absent in human plasma), the secreted carboxylesterase 1c in mouse plasma rapidly degrades the ester moiety of the prodrug before the compound enters cells. Therefore, for compounds with an ester prodrug moiety (such as SRI-43918, EIDD-2801, and Remdesivir), we may have to use *Ces1c*$^{-/-}$ mice for efficacy testing. To overcome this challenge, **we have obtained *Ces1c*$^{-/-}$ mice from Gilead**. Besides direct use for efficacy testing, the *Ces1c*$^{-/-}$ mice will be crossed with A129 or AG129 mice to create double knockout mice for DENV and/or ZIKV testing. The double knockout mice will be a critical reagent for the field of antiviral research.

B.2.d Screening effort for new dengue inhibitors. To ensure a robust flavivirus pipeline, we have initiated a new screen of 200,000 compounds. These compounds have not been tested in our previous screen. We will prioritize compounds with pan-DENV or pan-flavivirus activities. In addition, due to the sequence variations among the four serotypes of DENV, we plan to perform a pilot screen using DENV-4. This is because DENV-4 is more phylogenetically distant from DENV-2 that has been the only serotype for dengue drug discovery in the past two decades. We believe a DENV-4 screen may identify hits with different antiviral profiles.

Project 3 B.4 Training opportunities: All students, postdocs, and technicians have had the opportunity to learn real drug discovery through this project. These hands-on experiences are invaluable for their career development and future research selection. Besides performing experiments, they also participate in data analysis, project discussion, manuscript preparation, and presentation at conferences. These trainees are well positioned for potential industry career opportunities.

C. COMPONENT PRODUCTS

C.1 PUBLICATIONS

Not Applicable

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Not Applicable

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Not Applicable

C.5 OTHER PRODUCTS AND RESOURCE SHARING

Nothing to report

D. COMPONENT PARTICIPANTS

Not Applicable

E. COMPONENT IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

Not Applicable

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

NOTHING TO REPORT

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?
Not Applicable

F. COMPONENT CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. COMPONENT SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

Not Applicable

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Not Applicable

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT

Not Applicable

G.10 ESTIMATED UNOBLIGATED BALANCE

Not Applicable

G.11 PROGRAM INCOME

Not Applicable

G.12 F&A COSTS

Not Applicable

RESEARCH & RELATED BUDGET - SECTION A & B FINAL

ORGANIZATIONAL DUNS*: 0969975150000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: Oregon Health & Science University

Start Date*: 03-01-2020 **End Date*:** 02-28-2021

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		[redacted] (b) (6), (b) (3) (A)				Project Lead	(b) (6), (b) (4)	(b) (6)			18,700.00	4,675.00	23,375.00
2.	Dr					Co-investigator					35,057.00	8,764.00	43,821.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **67,196.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical	(b) (6)					
2	1 ResAsst. Prof/1 Research Associate				43,097.00	15,883.00	58,980.00
2	**Total Number Other Personnel**					**Total Other Personnel**	**58,980.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**126,176.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E

ORGANIZATIONAL DUNS*: 0969975150000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: Oregon Health & Science University

Start Date*: 03-01-2020 **End Date*:** 02-28-2021

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	1,500.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**1,500.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		0.00
2. Stipends		0.00
3. Travel		0.00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K

ORGANIZATIONAL DUNS*: 0969975150000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: Oregon Health & Science University

Start Date*: 03-01-2020 **End Date*:** 02-28-2021

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	31,082.00
2. Publication Costs	1,500.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
Total Other Direct Costs	**32,582.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**160,258.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. MTDC	54.0	160,258.00	86,539.00
		Total Indirect Costs	**86,539.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Arif M. Karim, 415-437-7820

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**246,797.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification* File Name: (b) (6), (b) (3) (A)_R&R 424 Budget U19AI142759_RPPR_Year 2_flat.pdf

(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - Budget Period 2

OMB Number: 4040-0001
Expiration Date: 10/31/2019

ORGANIZATIONAL DUNS: 0969975150000 **Enter name of Organization:** Oregon Health & Science University

Budget Type: ☐ Project ☒ Subaward/Consortium **Budget Period:** 2 **Start Date:** 03/07/2020 **End Date:** 02/28/2021

A. Senior/Key Person

Prefix	First	Middle	Last	Suffix	Base Salary ($)	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	(b) (6), (b) (3) (A)			PhD	(b) (6), (b) (4)	(b) (6)			18,700.00	4,675.00	23,375.00
Project Role:	PD/PI										
	(b) (6), (b) (3) (A)			PhD	(b) (6), (b) (4)	(b) (6)			35,057.00	8,764.00	43,821.00
Project Role:	Co-Investigator										

Additional Senior Key Persons: Add Attachment | Delete Attachment | View Attachment

Total Funds requested for all Senior Key Persons in the attached file:

Total Senior/Key Person 67,196.00

B. Other Personnel

Number of Personnel	Project Role	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Assistant Professor	(b) (6)			24,614.00	10,338.00	34,952.00
1	Research Associate				18,483.00	5,545.00	24,028.00

2 **Total Number Other Personnel**

Total Other Personnel 58,980.00

Total Salary, Wages and Fringe Benefits (A+B) 126,176.00

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment item	Funds Requested ($)

Additional Equipment: Add Attachment | Delete Attachment | View attachment

Total funds requested for all equipment listed in the attached file	
Total Equipment	

D. Travel

	Funds Requested ($)
1. Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	1,500.00
2. Foreign Travel Costs	
Total Travel Cost	1,500.00

E. Participant/Trainee Support Costs

	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other	
Number of Participants/Trainees	**Total Participant/Trainee Support Costs**

F. Other Direct Costs

		Funds Requested ($)
1.	Materials and Supplies	31,082.00
2.	Publication Costs	1,500.00
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.		
9.		
10.		
	Total Other Direct Costs	32,582.00

G. Direct Costs

	Funds Requested ($)
Total Direct Costs (A thru F)	160,258.00

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
MTDC	54.00	160,258.00	86,539.00

Total Indirect Costs	86,539.00

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Arif M. Karim, 415-437-7820

I. Total Direct and Indirect Costs

	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	246,797.00

J. Fee

Funds Requested ($)

K. Total Costs and Fee

	Funds Requested ($)
Total Costs and Fee (I + J)	246,797.00

L. Budget Justification

(Only attach one file.) Add Attachment | Delete Attachment | View Attachment

RESEARCH & RELATED BUDGET - Cumulative Budget

		Totals ($)
Section A, Senior/Key Person		67,196.00
Section B, Other Personnel		58,980.00
Total Number Other Personnel	2	
Total Salary, Wages and Fringe Benefits (A+B)		126,176.00
Section C, Equipment		
Section D, Travel		1,500.00
1. Domestic	1,500.00	
2. Foreign		
Section E, Participant/Trainee Support Costs		
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other		
6. Number of Participants/Trainees		
Section F, Other Direct Costs		32,582.00
1. Materials and Supplies	31,082.00	
2. Publication Costs	1,500.00	
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs		
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Other 1		
9. Other 2		
10. Other 3		
Section G, Direct Costs (A thru F)		160,258.00
Section H, Indirect Costs		86,539.00
Section I, Total Direct and Indirect Costs (G + H)		246,797.00
Section J, Fee		
Section K, Total Costs and Fee (I + J)		246,797.00

Notice of Award

RESEARCH
Department of Health and Human Services
National Institutes of Health

Federal Award Date: 08/04/2020

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Grant Number: 5R01AI132178-04
FAIN: R01AI132178

Principal Investigator(s):
(b) (6), (b) (3) (A) (contact), PHD
(b) (6), (b) (3) (A), PHD

Project Title: Broad-spectrum antiviral GS-5734 to treat MERS-CoV and related emerging CoV

(b) (6), (b) (3) (A)
104 Airport Drive
Suite (b) (6), (b) (3)
Chapel Hill, NC 27599

Award e-mailed to: resadminosr@unc.edu

Period Of Performance:
Budget Period: 08/01/2020 – 07/31/2021
Project Period: 08/09/2017 – 07/31/2022

Dear Business Official:

The National Institutes of Health hereby awards a grant in the amount of $1,166,670 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to UNIV OF NORTH CAROLINA CHAPEL HILL in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as "Research reported in this publication was supported by the National Institute Of Allergy And Infectious Diseases of the National Institutes of Health under Award Number R01AI132178. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health." Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination.

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator's Financial Conflict of Interest (FCOI), in accordance with the 2011 revised regulation at 42 CFR Part 50 Subpart F. The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module. The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards. Consult the NIH website http://grants.nih.gov/grants/policy/coi/ for a link to the regulation and additional important information.

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Sincerely yours,

NIH eGA (1) Version: 8.5 - 02/25/2018 3:12:00 PM Generated on: 08/04/2020 1:17:08 PM

Tina M. Carlisle
Grants Management Officer
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Additional information follows

NIH NGA R | Version: 56 - 12/26/2018 2:22:00 PM| Generated on: 8/5/2020 1:41:26 AM

SECTION I – AWARD DATA – 5R01AI132178-04

Award Calculation (U.S. Dollars)

Salaries and Wages	$154,747
Fringe Benefits	$46,236
Personnel Costs (Subtotal)	$200,983
Materials & Supplies	$220,895
Travel	$6,000
Other	$16,724
Subawards/Consortium/Contractual Costs	$471,000
Publication Costs	$2,000
Tuition Remission	$1,825
Federal Direct Costs	$919,427
Federal F&A Costs	$247,243
Approved Budget	$1,166,670
Total Amount of Federal Funds Obligated (Federal Share)	$1,166,670
TOTAL FEDERAL AWARD AMOUNT	$1,166,670
AMOUNT OF THIS ACTION (FEDERAL SHARE)	$1,166,670

SUMMARY TOTALS FOR ALL YEARS		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
4	$1,166,670	$1,166,670
5	$1,166,670	$1,166,670

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

Fiscal Information:

CFDA Name:	Allergy and Infectious Diseases Research
CFDA Number:	93.855
EIN:	1566001393A1
Document Number:	RAI132178A
PMS Account Type:	P (Subaccount)
Fiscal Year:	2020

IC	CAN	2020	2021
AI	8472315	$1,166,670	$1,166,670

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

NIH Administrative Data:
PCC: M51C B / **OC**: 41025 / **Released**: (b) (6) 08/04/2020
Award Processed: 08/05/2020 01:41:26 AM

SECTION II – PAYMENT/HOTLINE INFORMATION – 5R01AI132178-04

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III – TERMS AND CONDITIONS – 5R01AI132178-04

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

a. The grant program legislation and program regulation cited in this Notice of Award.
b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.
c. 45 CFR Part 75.

d. National Policy Requirements and all other requirements described in the NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
e. Federal Award Performance Goals: As required by the periodic report in the RPPR or in the final progress report when applicable.
f. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm for certain references cited above.)

Research and Development (R&D): All awards issued by the National Institutes of Health (NIH) meet the definition of "Research and Development" at 45 CFR Part§ 75.2. As such, auditees should identify NIH awards as part of the R&D cluster on the Schedule of Expenditures of Federal Awards (SEFA). The auditor should test NIH awards for compliance as instructed in Part V, Clusters of Programs. NIH recognizes that some awards may have another classification for purposes of indirect costs. The auditor is not required to report the disconnect (i.e., the award is classified as R&D for Federal Audit Requirement purposes but non-research for indirect cost rate purposes), unless the auditee is charging indirect costs at a rate other than the rate(s) specified in the award document(s).

This institution is a signatory to the Federal Demonstration Partnership (FDP) Phase VI Agreement which requires active institutional participation in new or ongoing FDP demonstrations and pilots.

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval.

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP).

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the System for Award Management (SAM). Should a consortium/subaward be issued under this award, a DUNS requirement must be included. See http://grants.nih.gov/grants/policy/awardconditions.htm for the full NIH award term implementing this requirement and other additional information.

This award has been assigned the Federal Award Identification Number (FAIN) R01AI132178. Recipients must document the assigned FAIN on each consortium/subaward issued under this award.

Based on the project period start date of this project, this award is likely subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170. There are conditions that may exclude this award; see http://grants.nih.gov/grants/policy/awardconditions.htm for additional award applicability information.

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: http://publicaccess.nih.gov/.

In accordance with the regulatory requirements provided at 45 CFR 75.113 and Appendix XII to 45 CFR Part 75, recipients that have currently active Federal grants, cooperative agreements, and procurement contracts with cumulative total value greater than $10,000,000 must report and maintain information in the System for Award Management (SAM) about civil, criminal, and administrative proceedings in connection with the award or performance of a Federal award that reached final disposition within the most recent five-year period. The recipient must also make semiannual disclosures regarding such proceedings. Proceedings information will be made publicly available in the designated integrity and performance system (currently the Federal

Awardee Performance and Integrity Information System (FAPIIS)). Full reporting requirements and procedures are found in Appendix XII to 45 CFR Part 75. This term does not apply to NIH fellowships.

Treatment of Program Income:
Additional Costs

SECTION IV – AI Special Terms and Conditions – 5R01AI132178-04

Clinical Trial Indicator: No
This award does not support any NIH-defined Clinical Trials. See the NIH Grants Policy Statement Section 1.2 for NIH definition of Clinical Trial.

This Notice of Award (NoA) includes funds for activity with **Vanderbilt University Medical Center**
****
This Notice of Award (NoA) includes funds for activity with **University of Texas Medical Branch.**
****
Awardees who conduct research involving Select Agents (see 42 CFR 73 for the Select Agent list; and 7 CFR 331 and 9 CFR 121 for the relevant animal and plant pathogens at http://www.selectagents.gov/Regulations.html) must complete registration with CDC (or APHIS, depending on the agent) before using NIH funds. No funds can be used for research involving Select Agents if the final registration certificate is denied.

Prior to conducting a restricted experiment with a Select Agent or Toxin, awardees must notify the NIAID and must request and receive approval from CDC or APHIS.
****
Select Agents:
Awardee of a project that at any time involves a restricted experiment with a select agent, is responsible for notifying and receiving prior approval from the NIAID. Please be advised that changes in the use of a Select Agent will be considered a change in scope and require NIH awarding office prior approval. The approval is necessary for new select agent experiments as well as changes in on-going experiments that would require change in the biosafety plan and/or biosafety containment level. An approval to conduct a restricted experiment granted to an individual cannot be assumed an approval to other individuals who conduct the same restricted experiment as defined in the Select Agents Regulation 42 CFR Part 73, Section 13.b
Highly Pathogenic Agent:
NIAID defines a Highly Pathogenic Agent as an infectious Agent or Toxin that may warrant a biocontainment safety level of BSL3 or higher according to the current edition of the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL)
Research funded under this grant must adhere to the BMBL, including using the BMBL-recommended biocontainment level at a minimum. If your Institutional Biosafety Committee (or equivalent body) or designated institutional biosafety official recommend a higher biocontainment level, the highest recommended containment level must be used. When submitting future Progress Reports indicate at the beginning of the report:

If no research with a Highly Pathogenic Agent or Select Agent has been performed or is planned to be performed under this grant.

If your IBC or equivalent body or official has determined, for example, by conducting a risk assessment, that the work being planned or performed under this grant may be conducted at a biocontainment safety level that is lower than BSL3.

If the work involves Select Agents and/or Highly Pathogenic Agents, also address the following points:

Any changes in the use of the Agent(s) or Toxin(s) including its restricted experiments that have resulted in a change in the required biocontainment level, and any resultant change in location, if applicable, as determined by your IBC or equivalent body or official.

If work with a new or additional Agent(s)/Toxin(s) is proposed in the upcoming project period, provide:

NIH eGA (1) Version: 86 - 12/26/2018 2:22:00 PM Generated on: 8/8/2019 11:08 AM

o A list of the new and/or additional Agent(s) that will be studied;
o A description of the work that will be done with the Agent(s), and whether or not the work is a restricted experiment;
o The title and location for each biocontainment resource/facility, including the name of the organization that operates the facility, and the biocontainment level at which the work will be conducted, with documentation of approval by your IBC or equivalent body or official. It is important to note if the work is being done in a new location.

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These individuals work together in overall project administration. Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

Grants Management Specialist: Tseday G Girma
Email: tseday.girma@nih.gov **Phone**: 240-747-7388 **Fax**: 301-493-0597

Program Official: Erik J. Stemmy
Email: erik.stemmy@nih.gov **Phone**: 240-627-3380

SPREADSHEET SUMMARY
GRANT NUMBER: 5R01AI132178-04

INSTITUTION: UNIV OF NORTH CAROLINA CHAPEL HILL

Budget	Year 4	Year 5
Salaries and Wages	$154,747	$154,747
Fringe Benefits	$46,236	$46,236
Personnel Costs (Subtotal)	$200,983	$200,983
Materials & Supplies	$220,895	$220,895
Travel	$6,000	$6,000
Other	$16,724	$16,724
Subawards/Consortium/Contractual Costs	$471,000	$471,000
Publication Costs	$2,000	$2,000
Tuition Remission	$1,825	$1,825
TOTAL FEDERAL DC	$919,427	$919,427
TOTAL FEDERAL F&A	$247,243	$247,243
TOTAL COST	$1,166,670	$1,166,670

Facilities and Administrative Costs	Year 4	Year 5
F&A Cost Rate 1	55.5%	55.5%
F&A Cost Base 1	$445,482	$445,482
F&A Costs 1	$247,243	$247,243

NIH eGA (1) Version: 5R- 12/26/2018 3:22:00 PM Generated on: 8/8/2019 1:17:18 PM

A. COVER PAGE

Project Title: Broad-spectrum antiviral GS-5734 to treat MERS-CoV and related emerging CoV	
Grant Number: 5R01AI132178-04	**Project/Grant Period:** 08/09/2017 - 07/31/2022
Reporting Period: 08/01/2019 - 07/31/2020	**Requested Budget Period:** 08/01/2020 - 07/31/2021
Report Term Frequency: Annual	**Date Submitted:** 06/19/2020
Program Director/Principal Investigator Information: (b) (6), (b) (3) (A) , PHD BS **Phone number:** (b) (6), (b) (3) (A) **Email:** (b) (6), (b) (3) (A)	**Recipient Organization:** UNIV OF NORTH CAROLINA CHAPEL HILL UNIVERSITY OF NORTH CAROLINA CHAPEL HILL Office of Sponsored Research CHAPEL HILL, NC 275990001 **DUNS:** 608195277 **EIN:** 1566001393A1 **RECIPIENT ID:**
Change of Contact PD/PI: N/A	
Administrative Official: (b) (6), (b) (3) (A) 104 Airport Dr. Suite 2200 Chapel Hill, NC 275991350 **Phone number:** (b) (6), (b) (3) (A) **Email:** (b) (6), (b) (3) (A)	**Signing Official:** (b) (6), (b) (3) (A) 104 Airport Drive Suite 2200 Chapel Hill, NC 27599 **Phone number:** (b) (6), (b) (3) (A) **Email:** (b) (6), (b) (3) (A)
Human Subjects: No	**Vertebrate Animals:** Yes
hESC: No	**Inventions/Patents:** No

B. ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

Aim 1: Pharmacokinetics and Pharmacodynamics of GS-5734. 1) Synthetically reconstruct group 2D CoV. 2) Determine if antiviral effect and drug metabolism are equivalent in various primary cells targeted by SARS- and MERS-CoV through measurement of TP levels, virus replication and toxicity. 3) Create a transgenic model for MERS-CoV efficacy studies and assess efficacy in young and aged mouse models of SARS- and MERS-CoV disease. 4) Assess efficacy of GS-5734 in non-human primate models of SARS- and MERS-CoV

Aim 2: Defining Resistance to GS-5734 and Impact on Replication, Pathogenesis and Treatment. 1) Select MERS-CoV GS-5734 resistance mutants in continuous and primary human airway cells, and in wild-type animals. 2) Determine the effect of passage-selected reverse- engineered GS-5734 resistance mutations on replication fidelity, viral RNA synthesis, and competitive fitness as compared to wild-type parental virus. 3) Determine if resistance mutations alter viral replication, pathogenesis, or treatment in vivo.

Aim 3: Defining the Mechanism of Action of GS-5734. 1) In cell culture, determine if GS-5734 alters SARS- and MERS-CoV RNA synthesis, sequence diversity, and the innate immune response. 2) In mice, determine if GS-5734 alters SARS- and MERS-CoV RNA synthesis, sequence diversity, and the innate immune response. 3) Use RNA FISH to determine how drug affects viral RNA replication and the host response.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: Accomplishments.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

For this reporting period, is there one or more Revision/Supplement associated with this award for which reporting is required?

Yes

Revision/ Supplements #	Revision/ Supplements Title	Specific Aims	Accomplishments
3R01AI132178-03S1		Aim 1: Defining the in vitro antiviral activity and in vivo efficacy of RDV against SARS-CoV-2. Aim 2: Defining Resistance to RDV and Impact on Replication, Pathogenesis and Treatment. Aim 3: Defining the Mechanism of Action of RDV.	This supplement is focused on better understanding the activity, efficacy and MOA of RDV against SARS-CoV-2 under the following specific aims: 1) Defining the in vitro antiviral activity and in vivo efficacy of RDV against SARS-CoV-2. 2) Defining Resistance to RDV and Impact on Replication, Pathogenesis and Treatment. 3) Defining the Mechanism of Action of RDV. Thus far, we have generated in vitro activity data in Vero, Calu-3 and HAE which is noted above in Figure 1. This work has already resulted in a manuscript that is under revision at Cell Reports.

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

File uploaded: Training.pdf

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

The results generated from this program have in part been disseminated via past publications (See below) and future publications of which we have one in revision currently. Results from this program have also been recently presented at the [redacted] (b) (6), (b) (3) (A)

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

In the next reporting period, we will continue to support the development of RDV to treat emerging CoV infections. First, we aim to recover a group 2D CoV recombinant viruses which will facilitate efficacy testing with RDV against this CoV subgenus. These viruses will further increase our ability to define the spectrum of antiviral activity against the CoV family. Second, we will identify the genetic pathways of MERS-CoV RDV resistance, engineering these mutations back into our infectious clone to confirm resistance phenotypes, assess effects on replicative fitness, pathogenesis and in vivo efficacy. Relatedly, we will further define the mechanism of RDV-mediated reduction in CoV SI through the quantification of full-length and truncated CoV RNA following treatment with RDV using MinION Nanopore long-read, single-molecule sequencing. Using similar technologies, we will also initiate studies to determine if RDV alteration of viral RNAs leads to changes in host viral RNA sensing or initiation of the innate immune response. This work described in Aim 3 of our initial application will help further define the MOA of RDV. Lastly, we will initiate NHP studies aimed at creating better models of emerging CoV that will facilitate the evaluation of antiviral spectrum breadth.

B.2 What was accomplished under these goals?

B.2.1. Overview of Major Activities and Industry Engagement. Since the initiation of this Partnership Grant, we have had monthly calls with [redacted] and the academic team. Through their consistent engagement in meetings/conference calls, reagent development/accessibility and the funding of work prior to this award, [redacted] has repeatedly demonstrated their commitment to this program. For the past 5 months, the frequency of our communication with [redacted] has increased to address the COVID-19 pandemic. As in the past, we continue to generate data important for the progression of RDV to treat coronavirus and better understand the biology of this drug.

B.2.2. Specific Objectives for Year 3. 1) Synthetically reconstruct group 2D CoV and new enzootic CoV 2) Determine the antiviral activity spectrum against human and zoonotic CoV, 3) Mechanism of action studies and resistance generation, 4) In vivo efficacy studies and 5) NHP model development.

B.2.3. Significant Results for Year 3.

1. Synthetically reconstruct group 2D CoV and new enzootic CoV. The coronavirus family can be divided into four genera (1 (alpha), 2 (beta), 3 (gamma), 4 (delta)) and the 2D subgenus thus far only contains bat CoV. Given the proclivity for bat CoV to emerge, we aim to determine if RDV is efficacious against group 2D CoV. In the funding period, we have designed, ordered and obtained two infectious cDNA clones based on complete viral genome sequences isolated from fruit bats (*Rousettus leschenaulti*) in China. We have evidence of genomic RNA replication by the detection of leader-containing transcripts for one of these genomes and are currently trouble shooting recovery of recombinant virus. Once characterized, antiviral assays will be developed for these viruses in order to determine the RDV antiviral activity against group 2D CoV. In addition, we are currently designing infectious clones for pangolin CoV (GSAID Accession EPI_ISL_410721) and bat CoV RaTG13 (Accession # MN996532).

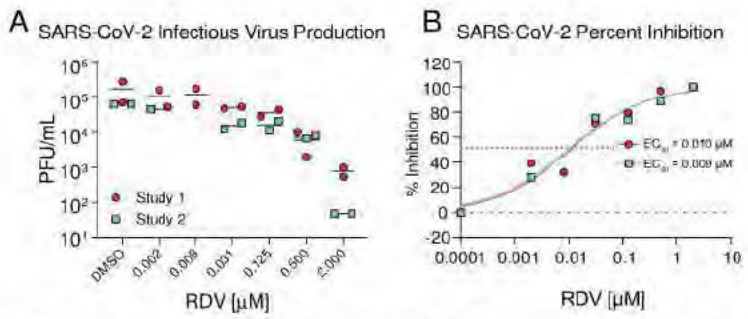


Figure 1: RDV is potently antiviral against SARS-CoV-2 in primary human airway epithelial (HAE) cell cultures. HAE were infected at an MOI of 0.5 with SARS-CoV-2 clinical isolate from CDC in the presence of a dose response of RDV. Infectious virus production was measured by plaque assay of apical washes at 48hr post infection.

2. Determine antiviral activity spectrum against human and zoonotic CoV. Since the origination of this program, we have evaluated the antiviral activity of RDV against many human and zoonotic CoV including SARS-CoV, MERS-CoV, mouse hepatitis virus (MHV), human CoVs OC43 (HCoV-OC43), 229E (HCoV-229E) and NL663 (HCoV-NL63) and emerging porcine CoV porcine deltacoronavirus (PDCoV). In the last funding period, we published a paper describing activity against HCoV-OC43 and 229E as well as PDCoV (2019 Brown et al.). PDCoV has the most divergent RdRp of known CoV as compared to SARS-CoV and MERS-CoV (67-69% amino acid identity). Thus, RDV remains active even when the target of the mechanism of action, the RNA-dependent RNA polymerase, is variable. In addition, we have performed studies demonstrating that RDV is potently active against pandemic SARS-CoV-2 in primary human airway epithelial cell cultures (**Fig. 1**). In summary, we have thoroughly defined the spectrum of RDV's antiviral activity against human endemic, epidemic and pandemic CoV as well as multiple zoonotic CoV and will continue to further evaluate spectrum as we construct new CoV.

3. Mechanism of action studies and resistance generation.

MERS-CoV passage for resistance. To define the mechanism of action of RDV against CoV we have been pursuing complementary approaches at UNC and Vanderbilt University Medical Center (VUMC). At UNC, we have passaged MERS-CoV in human primary airway epithelial (HAE) cultures in the presence of either high (1μM) or low (0.25μM) RDV. Upon quantitation of MERS-CoV titers from each passage, we found by passage 18, MERS-CoV titers were increased over 2 logs from those observed in passage 1. In collaboration with Vanderbilt we aim to deep sequence the virus populations over time to define putative resistance mutations that may be driving enhanced replication in the presence of RDV. These data will be compared to those we are currently generating for SARS-CoV-2 and those for MHV. The goal is to determine if a variety of CoV evolve similar or different pathways to RDV.

Nsp13 mutation contributes to remdesivir resistance. Our previous work showed that serial passage of MHV in the presence of remdesivir yielded two mutations in the nsp12-RdRp, F476L and V553L, that together conferred a level of resistance to RDV approaching the passage-virus population (p23), yet did not completely recapitulate the phenotype (Agostini et al., 2018). To determine if a co-occurring A335V mutation in the nsp13-helicase gene contributes to RDV resistance, we tested this mutation alone and combined with the two RdRp mutations in an isogenic WT MHV background for sensitivity to RDV. Our results demonstrate that nsp13-A335V contributes to RDV resistance and the combination of nsp12-F476L/V553L and nsp13-A335V recapitulates the resistance level observed in the p23 virus population (**Fig. 2**). These data indicate that alterations in replicase proteins other than the viral polymerase can affect RDV sensitivity, which has important treatment implications. Upcoming studies will determine effects nsp12 and nsp13 resistance mutations in pre-zoonotic CoV strains.

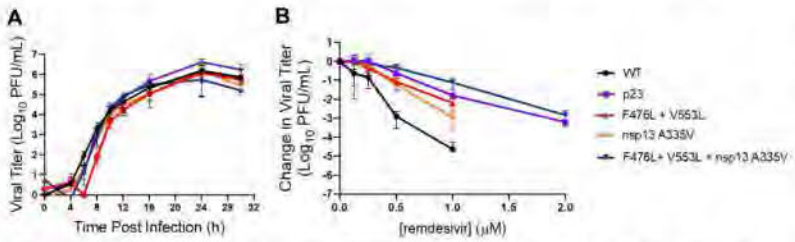


Figure 2: Nsp13 A335V contributes to remdesivir resistance in MHV. (A) Replication of recombinant MHV strains in DBT9 cells. (B) Effect of remdesivir on viral titer of MHV-nsp13-A335V and nsp12 F481L/V553L alterations alone and in combination versus wild-type (WT) and population virus passaged for remdesivir resistance (p23).

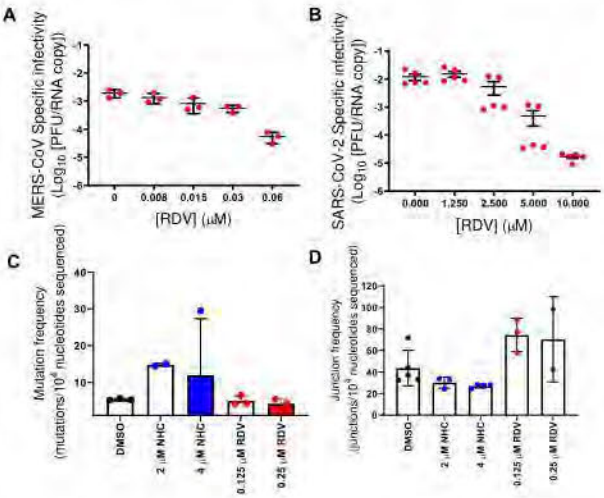


Figure 3: Reduction in specific infectivity following remdesivir (RDV) treatment is not caused by mutagenesis or increased defective genome production. (A) Specific infectivity of SARS-CoV-2 in Calu3 and (B) MERS-CoV in Huh7 cells. (C, D) Effect of RDV and NHC on MHV mutation frequency and junction frequency.

Reduction in specific infectivity of RDV-treated CoV likely occurs through a novel mechanism. We previously demonstrated that treatment of MHV- infected cells with RDV results in decreases in genome copy number, infectious virus production, and ratio of infectious virus particles to viral genomes (specific infectivity [SI]). We now report that SI of MERS-CoV and SARS-CoV-2 is also decreased in a dose-dependent fashion following treatment with RDV (**Fig. 3A, B**), which suggests that RDV-associated reductions in SI involves conserved determinants or functions across diverse CoV. Because a decrease in SI has been linked to drugs that inhibit viral replication by mutagenesis, we used deep-sequencing to determine MHV mutation frequency in supernatant virion particles released RDV-treated cultures. We included β-D-*N*4-hydroxycytidine (NHC), a known mutagen and potent CoV inhibitor, as a control. Our results show that although NHC treatment causes an expected dose-dependent increase in mutation frequency, treatment with RDV does not (**Fig. 3C**). These results raise the possibility that RDV promotes generation of defective viral genomes (DVGs). We tested this hypothesis by quantifying recombination junction frequency using CoV next-generation sequencing pipelines previously established in our lab. Junction frequency was not significantly altered by exposure to high doses of either RDV or NHC (**Fig. 3D**), indicating that neither RDV nor NHC drives increased DVG formation. This outcome is quite exciting as it suggests a novel mechanism of antiviral action by RDV and impaired fitness (presumably translating to reduced transmission) of viral progeny. The mechanism of RDV-mediated reduction in CoV SI will be explored in the upcoming funding period, to include quantification of full-length and truncated CoV RNA following treatment with RDV using MinION Nanopore long-read, single-molecule sequencing.

4. In vivo efficacy studies. In the previous funding period, we completed studies demonstrating the in vivo efficacy of RDV against MERS-CoV [redacted] et. al Nature Communications). In these studies, we performed several head to head therapeutic efficacy studies comparing RDV against lopinavir/ritonavir and interferon beta in our MERS-CoV model. We showed that only RDV could reduce virus titer. We also developed a quantitative scoring system for pathological features of acute lung injury (ALI) in collaboration with a veterinary pathologist, [redacted]. We applied a system developed by the American Thoracic Society to our viral pathogenesis models. Using this tool, we showed that only RDV could significantly reduce the pathological features or ALI in our MERS model (**Fig. 4**) . We have since used this tool to demonstrate that another nucleoside analog EIDD-2801 could reduce ALI in a SARS-CoV mouse model. Lastly, to rapidly assess the potential for RDV to treat SARS-CoV-2 infections, we created a chimeric SARS-CoV virus encoding the SARS-CoV-2 RdRp. In mice, we demonstrated that therapeutic RDV could significantly reduce viral loads and improve pulmonary function in mice infected with this chimeric virus. A manuscript detailing in vitro studies with RDV and SARS-CoV-2 and in vivo studies with the above noted chimeric virus is currently under review at Cell Reports. Together, in the previous funding period, we have demonstrated in vivo efficacy of RDV against MERS-CoV and have performed the initial studies suggesting that RDV will be efficacious against SARS-CoV-2. These studies have in part enabled the human clinical trials for RDV to treat COVID-19.

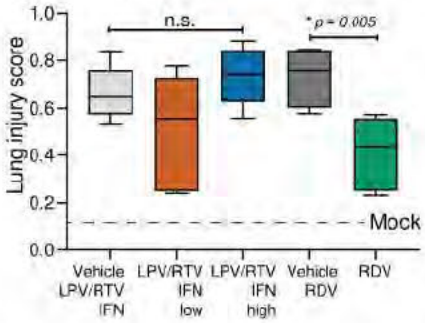


Figure 4: RDV diminishes the pathological features of acute lung injury in mice infected with MERS-CoV. The histological features of acute lung injury were blindly scored for neutrophils in the alveolar and interstitial space, hyaline membranes, proteinaceous debris filling the air spaces, and alveolar septal thickening. Three randomly chosen high power (×60) fields of diseased lung were assessed per mouse.

5. Non-human primate (NHP) model development. Non-human primate (NHP) model development. [redacted], an investigator at the University of Texas Medical Branch and Galveston National Laboratories (GNL), is leading our NHP efficacy efforts. [redacted] has recently performed MERS-CoV prophylactic and therapeutic studies in rhesus macaques at NIAID [redacted], thus completing the essential NHP efficacy studies for RDV preclinical development. There are no NHP models for zoonotic emerging CoVs similar to SARS-CoV (SCH014, WIV1) or MERS (HKU5, HKU4 etc.). Similarly, there are no NHP models for newly emerging pathogens like swine acute diarrhea syndrome (SADS-CoV) which have unknown epidemic potential in humans. An IACUC protocol for initiating a pilot study in NHPs was approved in late 2019 whereas the MTA enabling us to receive SARD-CoV from UNC has also been executed in February, 2020. For the next year, we will aim to design and execute the study of SADS-CoV infection in NHPs based on the established strategies for detecting the infection and disease (if any).

B.2.4. Key Outcomes or Other Achievements. The preclinical development of this program played a significant role in the origination of human clinical trials for RDV in China and in the United States. In addition, the past preclinical work supported by this program as well as very recent SARS-CoV-2 work has helped support recent FDA approval RDV to treat COVID-19. This work has also led to publication of several high impact papers.

B.4 What opportunities for training and professional development has the project provided?
Postdoctoral fellows and graduate students are active in the project. Individual development plans (IDPs) are generated on an annual basis. They are used for defining key objectives and goals for progress and for review on at least an annual basis. For this project, the IDPs will review specific goals relevant to the project. In addition, for postdoctoral fellows they aid in career development and through generating biosketches and CVs.

C. PRODUCTS

C.1 PUBLICATIONS

Are there publications or manuscripts accepted for publication in a journal or other publication (e.g., book, one-time publication, monograph) during the reporting period resulting directly from this award?

Yes

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Nothing to report

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Have inventions, patent applications and/or licenses resulted from the award during the reporting period? No

If yes, has this information been previously provided to the PHS or to the official responsible for patent matters at the grantee organization?

C.5 OTHER PRODUCTS AND RESOURCE SHARING

Category	Explanation
Models	We have developed the Ces1c-/- hDPP4 mouse model for the in vivo efficacy testing of GS-5734 with MERS-CoV. This model development was paid for by (b) (6), (b) (3) (A) and performed at (b) (6), (b) (3) (A).

D. PARTICIPANTS

D.1 WHAT INDIVIDUALS HAVE WORKED ON THE PROJECT?

Commons ID	S/K	Name	Degree(s)	Role	Cal	Aca	Sum	Foreign Org	Country	SS
(b) (6)	Y	(b) (6), (b) (3) (A)	BS,PHD	PD/PI	(b) (6)	0.0	0.0			NA
	Y		BS,PHD	PD/PI		0.0	0.0			NA
	N			Technician		0.0	0.0			NA
	N			Non-Student Research Assistant		0.0	0.0			NA
	N		BS	Technician		0.0	0.0			NA
	N			Technician		0.0	0.0			NA
	N			Technician		0.0	0.0			NA
	N		BA	Technician		0.0	0.0			NA
	N		BS,PHD	Co-Investigator		0.0	0.0			NA
	N		PHD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position		0.0	0.0			NA
	N			Technician		0.0	0.0			NA
	N		PHD,MS	Staff scientist (Doctoral level)		0.0	0.0			NA
	N			Technician		0.0	0.0			NA
	N		PHD,MD, MS,BS	Co-Investigator		0.0	0.0			NA
	N	DENISON, MARK R.	MD	Co-Investigator		0.0	0.0			NA
	N	(b) (6), (b) (3) (A)	BS	Graduate Student (research assistant)		0.0	0.0			NA
	N		MS,PHD	Co-Investigator		0.0	0.0			NA
	N		BS,PHD	Staff scientist (Doctoral level)		0.0	0.0			NA
	N		PHD	Postdoctoral		0.0	0.0			NA

(b) (6)		(b) (6), (b) (3) (A)		Scholar, Fellow, or Other Postdoctoral Position	(b) (6)				
	N			Non-Student Research Assistant	0.0	0.0			NA
	N		PHD,MS	Co-Investigator	0.0	0.0			NA
	N		PHD	Co-Investigator	0.0	0.0			NA

Glossary of acronyms:
S/K - Senior/Key
DOB - Date of Birth
Cal - Person Months (Calendar)
Aca - Person Months (Academic)
Sum - Person Months (Summer)
Foreign Org - Foreign Organization Affiliation
SS - Supplement Support
RE - Reentry Supplement
DI - Diversity Supplement
OT - Other
NA - Not Applicable

D.2 PERSONNEL UPDATES

D.2.a Level of Effort

Will there be, in the next budget period, either (1) a reduction of 25% or more in the level of effort from what was approved by the agency for the PD/PI(s) or other senior/key personnel designated in the Notice of Award, or (2) a reduction in the level of effort below the minimum amount of effort required by the Notice of Award?

No

D.2.b New Senior/Key Personnel

Are there, or will there be, new senior/key personnel?

No

D.2.c Changes in Other Support

Has there been a change in the active other support of senior/key personnel since the last reporting period?

Yes

File uploaded: Combined_OS.pdf

D.2.d New Other Significant Contributors

Are there, or will there be, new other significant contributors?

No

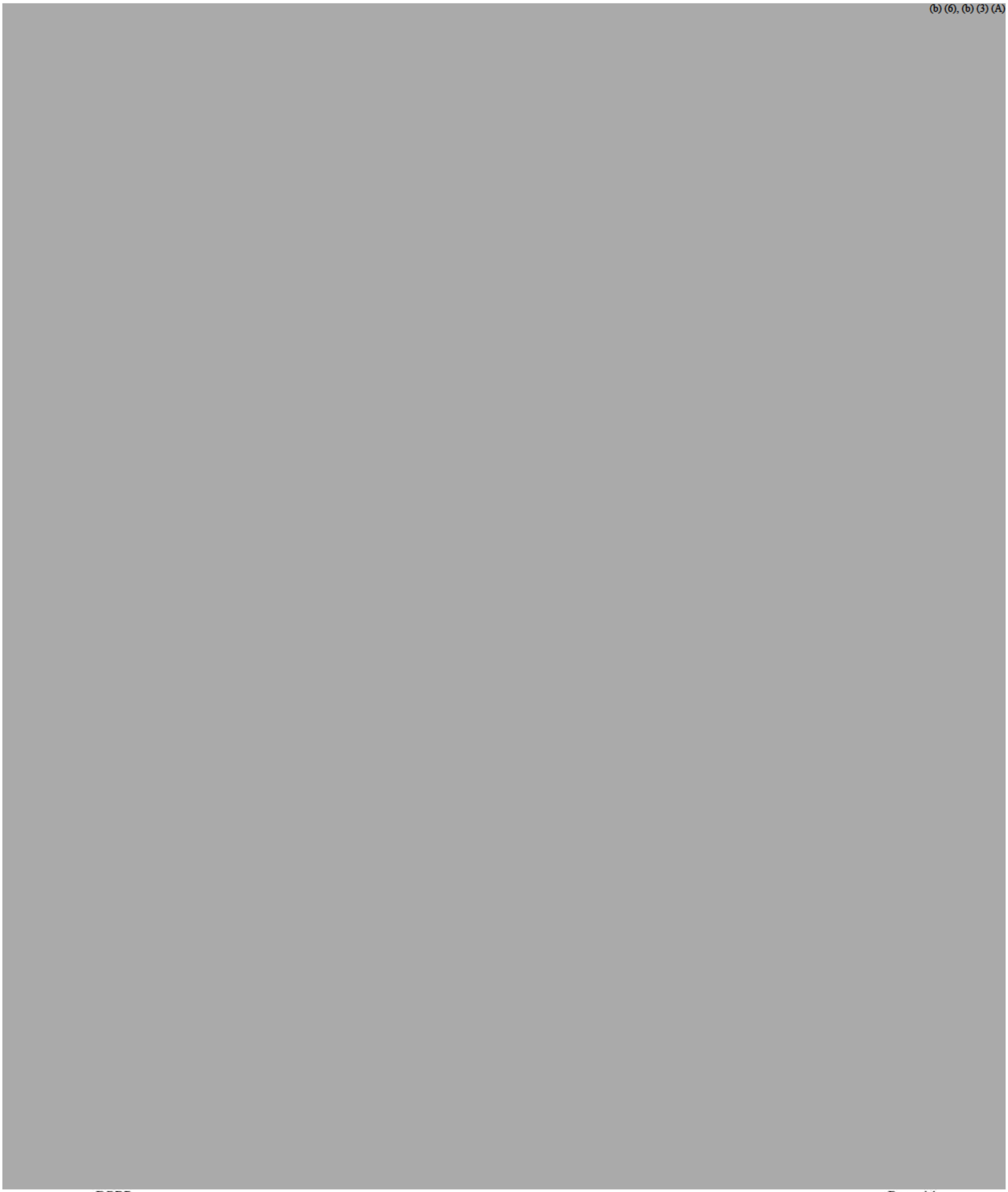
D.2.e Multi-PI (MPI) Leadership Plan

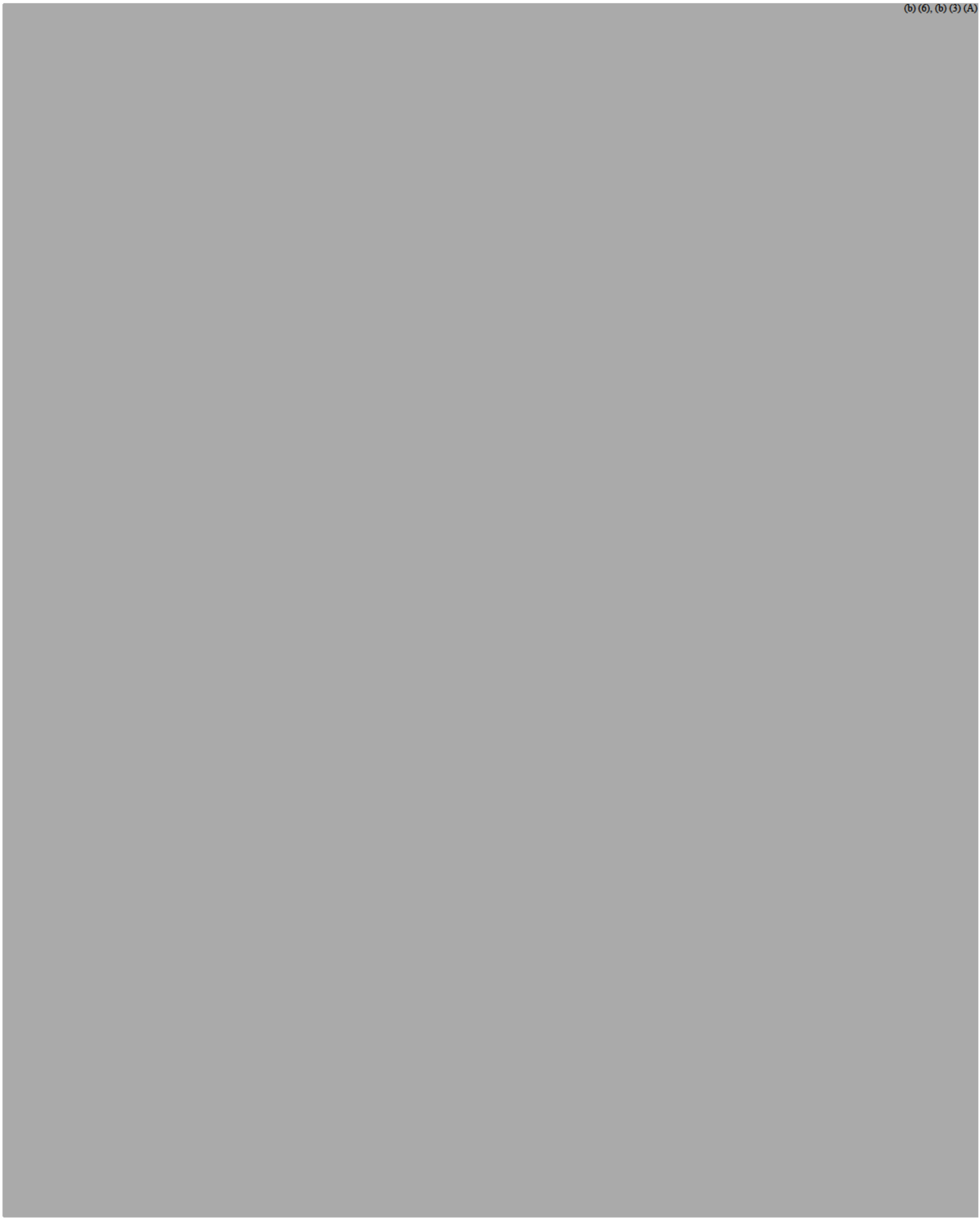
Will there be a change in the MPI Leadership Plan for the next budget period?

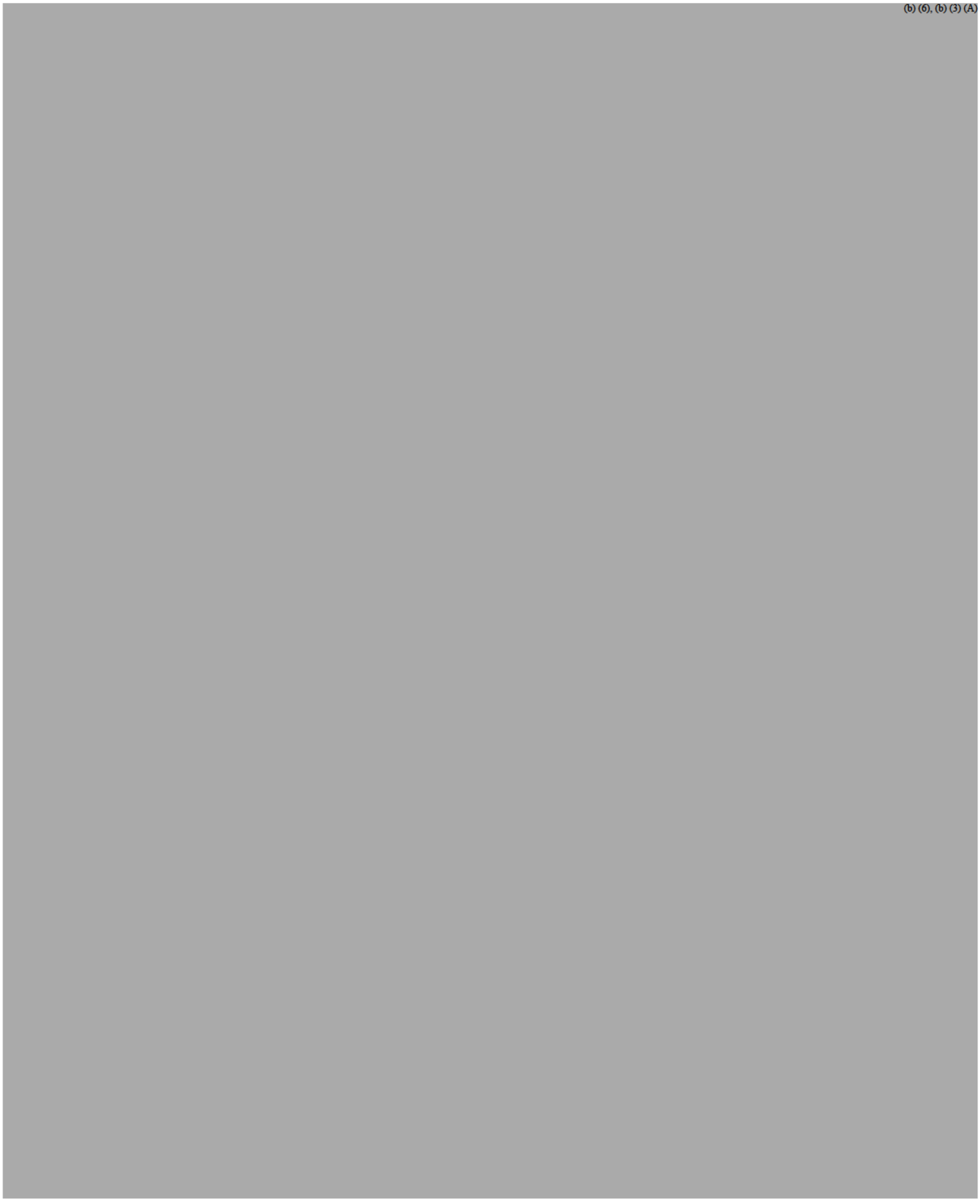
NA

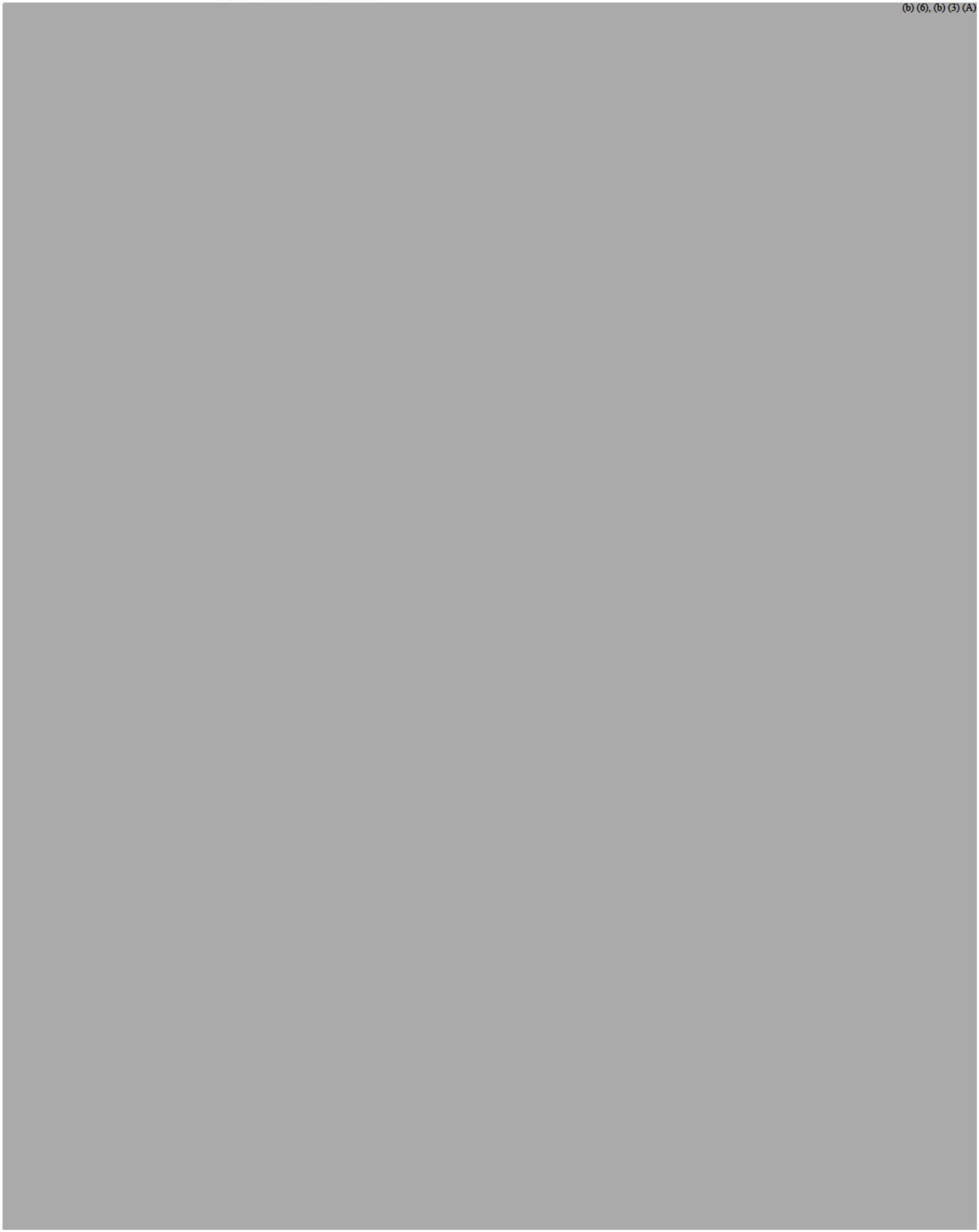
OTHER SUPPORT

(b) (6), (b) (3) (A)




(b) (6), (b) (3) (A)


(b) (6), (b) (3) (A)


(b) (6), (b) (3) (A)

(b) (6), (b) (3) (A)

[redacted]

PENDING:

(b) (6), (b) (3) (A), (b) (4)

[redacted]

(b) (6), (b) (3) (A), (b) (4)

OTHER SUPPORT

(b) (6), (b) (3) (A)

ACTIVE:

(b) (6), (b) (3) (A)

(b) (6), (b) (3) (A)

[redacted]

PENDING:

(b) (6), (b) (3) (A), (b) (4)

[redacted]

E. IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

NOTHING TO REPORT

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

Not Applicable

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?

NOTHING TO REPORT

F. CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

NOTHING TO REPORT

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Are there personnel on this project who are newly involved in the design or conduct of human subjects research?

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Does this project involve vertebrate animals?

Yes

G.8 PROJECT/PERFORMANCE SITES

Organization Name:	DUNS	Congressional District	Address
Primary: The University of North Carolina at Chapel Hill	608195277	NC-004	(b) (6), (b) (3) (A)
Vanderbilt University Medical Center	079917897	TN-005	
University of Texas Medical Branch	800771149	TX-014	

G.9 FOREIGN COMPONENT

No foreign component

G.10 ESTIMATED UNOBLIGATED BALANCE

G.10.a Is it anticipated that an estimated unobligated balance (including prior year carryover) will be greater than 25% of the current

year's total approved budget?

No

G.11 PROGRAM INCOME

Is program income anticipated during the next budget period?

No

G.12 F&A COSTS

Is there a change in performance sites that will affect F&A costs?

No

OMB Approval 2700-0042

AWARD/CONTRACT	1. THIS CONTRACT IS A RATED ORDER UNDER DPAS (15 CFR 350) ▶	RATING	PAGE	OF	PAGES
		N/A	1		46

2. CONTRACT (Proc. Inst. Ident.) NO.	3. EFFECTIVE DATE	4. REQUISITION/PURCHASE REQUEST/PROJECT NO.
75N93019C00058	September 9, 2019	5489284

5. ISSUED BY	CODE	6. ADMINISTERED BY (If other than Item 6)	CODE
National Institutes of Health National Institute of Allergy and Infectious Diseases DEA, Office of Acquisitions 5601 Fishers Lane, Room 3D37, MSC 9821 Bethesda, Maryland 20892-9821		MID RCB-A	N/A

7. NAME AND ADDRESS OF CONTRACTOR (No. street, county, state and ZIP Code)	8. DELIVERY	9. DISCOUNT FOR PROMPT PAYMENT
Emory University Office of Sponsored Programs 1599 Clifton Road, NE, 4th Floor Atlanta, GA 30322-4250	☐ FOB ORIGIN ☒ OTHER (See below) FOB Destination	N/A

CODE	FACILITY CODE	10. SUBMIT INVOICES ADDRESS SHOWN IN:	ITEM
			Art. G.3

11. SHIP TO/MARK FOR	CODE	12. PAYMENT WILL BE MADE BY	CODE
See Section E.	N/A	See Article G.3	N/A

13. AUTHORITY FOR USING OTHER FULL AND OPEN COMPETITION:
☐ 10 U.S.C. 2304(c)( ) ☐ 41 U.S.C. 253(c)(1)

ACCOUNTING AND APPROPRIATION DATA:
CAN: 19-8470036 Obligate: $1,637,319

15A. ITEM NO.	15B. SUPPLIES/SERVICES	15C. QUANTITY	15D. UNIT	15E. UNIT PRICE	15F. AMOUNT
	Title: The Development of EIDD-2801, the 5'-Isopropylester Prodrug of the Broadly Active Antiviral Ribonucleoside Analog EIDD-9131, for the Treatment of Influenza Infections **Period of Performance:** September 9, 2019 through March 8, 2020 **Contract Type:** Cost Reimbursement - completion	Base Period			$1,637,319

15G. TOTAL AMOUNT OF CONTRACT ▶ $1,637,319

16. TABLE OF CONTENTS

(✓)	SEC	DESCRIPTION	PAGE(S)	(✓)	SEC	DESCRIPTION	PAGE(S)
		PART I - THE SCHEDULE				PART II - CONTRACT CLAUSES	
☒	A	CONTRACT FORM	1	☒	I	CONTRACT CLAUSES	37
☒	B	SERVICES AND COST	5			PART III - LIST OF DOCUMENTS, EXHIBITS AND OTHER ATTACH.	
☒	C	DESCRIPTION/SPECS./WORK STATEMENT	6	☒	J	LIST OF ATTACHMENTS	45
☒	D	PACKAGING AND MARKING	12			PART IV - REPRESENTATIONS AND INSTRUCTIONS	
☒	E	INSPECTION AND ACCEPTANCE	12	☒	K	REPRESENTATIONS, CERTIFICATIONS AND OTHER STATEMENTS OF OFFERORS	46
☒	F	DELIVERIES OR PERFORMANCE	12				
☒	G	CONTRACT ADMINISTRATION DATA	14	☐	L	INSTRS., CONDS., AND NOTICES TO OFFERORS	
☒	H	SPECIAL CONTRACT REQUIREMENTS	18	☐	M	EVALUATION FACTORS FOR AWARD	

CONTRACTING OFFICER WILL COMPLETE ITEM 17 OR 18 AS APPLICABLE

17. ☒ CONTRACTOR'S NEGOTIATED AGREEMENT
(Contractor is required to sign this document and return one electronic copy to issuing office.) Contractor agrees to furnish and deliver all items or perform all the services set forth or otherwise identified above and on any continuation sheets for the consideration stated herein. The rights and obligations of the parties to this contract shall be subject to and governed by the following documents: (a) this award/contract, (b) the solicitation, if any, and (c) such provisions, representations, certifications, and specifications, as are attached or incorporated by reference herein. *(Attachments are listed herein.)*

18. ☐ AWARD *(Contractor is not required to sign this document.)* Your offer on Solicitation Number _____, including the additions or changes made by you which additions or changes are set forth in full above, is hereby accepted as to the items listed above and on any continuation sheets. This award consummates the contract which consists of the following documents: (a) the Government's solicitation and your offer, and (b) this award/contract. No further contractual document is necessary.

19A. NAME AND TITLE OF SIGNER *(Type or print)*	20A. NAME OF CONTRACTING OFFICER
Janette Hannam Hayes Associate Director for Research	Swee L. Teo Contracting Officer, MID RCB-A, OA,DEA,NIAID

19B. NAME OF CONTRACTOR	19C. DATE SIGNED	20B. UNITED STATES OF AMERICA	20C. DATE SIGNED
(b) (6)	9/5/19	BY (b) (6)	09/06/2019
(Signature of person authorized to sign)		*(Signature of Contracting Officer)*	

CONTRACT TABLE OF CONTENTS

PART I - THE SCHEDULE . . . 5
SECTION B - SUPPLIES OR SERVICES AND PRICES/COSTS . . . 5
ARTICLE B.1. BRIEF DESCRIPTION OF SERVICES . . . 5
ARTICLE B.2. ESTIMATED COST - OPTION . . . 5
ARTICLE B.3. ADVANCE UNDERSTANDINGS . . . 5
ARTICLE B.4. PROVISIONS APPLICABLE TO DIRECT COSTS . . . 5
SECTION C - DESCRIPTION/SPECIFICATIONS/WORK STATEMENT . . . 6
ARTICLE C.1. STATEMENT OF WORK . . . 7
ARTICLE C.2. REPORTING REQUIREMENTS . . . 7
ARTICLE C.3. INVENTION REPORTING REQUIREMENT . . . 11
SECTION D - PACKAGING, MARKING AND SHIPPING . . . 12
SECTION E - INSPECTION AND ACCEPTANCE . . . 12
SECTION F - DELIVERIES OR PERFORMANCE . . . 12
ARTICLE F.1. COMPLETION DATE . . . 12
ARTICLE F.2. DELIVERIES . . . 13
ARTICLE F.3. CLAUSES INCORPORATED BY REFERENCE, FAR 52.252-2 (FEBRUARY 1998) . . . 14
SECTION G - CONTRACT ADMINISTRATION DATA . . . 14
ARTICLE G.1. CONTRACTING OFFICER'S REPRESENTATIVE (COR) . . . 15
ARTICLE G.2. KEY PERSONNEL, HHSAR 352.237-75 (December 2015) . . . 15
ARTICLE G.3. INVOICE SUBMISSION/CONTRACT FINANCING REQUEST AND CONTRACT FINANCIAL REPORT . . . 15
ARTICLE G.4. PROVIDING ACCELERATED PAYMENT TO SMALL BUSINESS SUBCONTRACTORS, FAR 52.232-40 (December 2013) . . . 17
ARTICLE G.5. INDIRECT COST RATES . . . 17
ARTICLE G.6. POST AWARD EVALUATION OF CONTRACTOR PERFORMANCE . . . 18
SECTION H - SPECIAL CONTRACT REQUIREMENTS . . . 18
ARTICLE H.1. PROTECTION OF HUMAN SUBJECTS, HHSAR 352.270-4(b) (December 2015) . . . 18
ARTICLE H.2. HUMAN SUBJECTS . . . 19
ARTICLE H.3. RESTRICTION ON USE OF HUMAN SUBJECTS, HHSAR 352.270-6 (December 2015) . . . 19
ARTICLE H.4. REQUIRED EDUCATION IN THE PROTECTION OF HUMAN RESEARCH PARTICIPANTS . . . 19
ARTICLE H.5. DATA AND SAFETY MONITORING IN CLINICAL TRIALS . . . 20
ARTICLE H.6. GOOD CLINICAL PRACTICE TRAINING FOR NIH AWARDEES INVOLVED IN NIH-FUNDED CLINICAL TRIALS . . . 20
ARTICLE H.7. CLINICAL TRIAL REGISTRATION AND RESULTS INFORMATION SUBMISSION . . . 20
ARTICLE H.8. CLINICAL TRIAL REGISTRATION AND RESULTS INFORMATION SUBMISSION PLAN . . . 21
ARTICLE H.9. CERTIFICATE OF CONFIDENTIALITY . . . 22
ARTICLE H.10. SINGLE INSTITUTIONAL REVIEW BOARD (sIRB) . . . 23
ARTICLE H.11. PLAN FOR SINGLE INSTITUTIONAL REVIEW BOARD (sIRB) . . . 24
ARTICLE H.12. INCLUSION OF WOMEN AND MINORITIES IN RESEARCH INVOLVING HUMAN SUBJECTS 25
ARTICLE H.14. POSTING CLINICAL TRIAL INFORMED CONSENT FORMS TO CLINICALTRIALS.GOV . . . 26
ARTICLE H.15. REGISTRATION AND RESULTS REPORTING FOR APPLICABLE CLINICAL TRIALS CLINICALTRIALS.GOV . . . 26

ARTICLE H.16. HUMAN MATERIALS. 27
ARTICLE H.17. HUMAN MATERIALS (ASSURANCE OF OHRP COMPLIANCE). 27
ARTICLE H.20. SHARING HeLa CELL WHOLE GENOME SEQUENCE DATA AND FAMILY ACKNOWLEDGEMENT. 28
ARTICLE H.21. NIH POLICY ON ENHANCING PUBLIC ACCESS TO ARCHIVED PUBLICATIONS RESULTING FROM NIH-FUNDED RESEARCH. 28
ARTICLE H.22. NEEDLE EXCHANGE, HHSAR 352.270-12 (December 2015). 29
ARTICLE H.23. ACKNOWLEDGEMENT OF FEDERAL FUNDING. 29
ARTICLE H.24. CONTINUED BAN ON FUNDING ABORTION AND CONTINUED BAN ON FUNDING OF HUMAN EMBRYO RESEARCH, HHSAR 352.270-13 (December 2015). 29
ARTICLE H.25. LIMITATION ON USE OF FUNDS FOR PROMOTION OF LEGALIZATION OF CONTROLLED SUBSTANCES. 29
ARTICLE H.26. DISSEMINATION OF FALSE OR DELIBERATELY MISLEADING INFORMATION. 29
ARTICLE H.27. CARE OF LIVE VERTEBRATE ANIMALS, HHSAR 352.270-5(b) (December 2015). 30
ARTICLE H.28. ANIMAL WELFARE. 30
ARTICLE H.29. INTRODUCTION OF RODENTS AND RODENT PRODUCTS. 30
ARTICLE H.30. PROTECTION OF PERSONNEL WHO WORK WITH NONHUMAN PRIMATES. 31
ARTICLE H.31. RESTRICTION FROM USE OF LIVE VERTEBRATE ANIMALS. 31
ARTICLE H.32. OMB CLEARANCE. 31
ARTICLE H.33. RESTRICTION ON PORNOGRAPHY ON COMPUTER NETWORKS. 31
ARTICLE H.34. GUN CONTROL. 31
ARTICLE H.35. OPTION PROVISION. 31
ARTICLE H.36. SUBCONTRACTING PROVISIONS. 31
ARTICLE H.37. ELECTRONIC AND INFORMATION TECHNOLOGY ACCESSIBILITY, HHSAR 352.239-74 (December 2015). 32
ARTICLE H.38. INSTITUTIONAL RESPONSIBILITY REGARDING INVESTIGATOR FINANCIAL CONFLICTS OF INTEREST. 33
ARTICLE H.39. PUBLICATION AND PUBLICITY. 35
ARTICLE H.40. REPORTING MATTERS INVOLVING FRAUD, WASTE AND ABUSE. 35
ARTICLE H.41. OBTAINING AND DISSEMINATING BIOMEDICAL RESEARCH RESOURCES. 36
ARTICLE H.42. SHARING RESEARCH DATA. 36
ARTICLE H.43. POSSESSION USE AND TRANSFER OF SELECT BIOLOGICAL AGENTS OR TOXINS. . 36
ARTICLE H.44. PROHIBITION ON CONTRACTOR INVOLVEMENT WITH TERRORIST ACTIVITIES. 37
ARTICLE H.45. CONSTITUTION DAY. 37
ARTICLE H.46. USE OF FUNDS FOR CONFERENCES, MEETINGS AND FOOD. 37
PART II - CONTRACT CLAUSES. 37
SECTION I - CONTRACT CLAUSES. 37
PART III - LIST OF DOCUMENTS, EXHIBITS AND OTHER ATTACHMENTS. 45
SECTION J - LIST OF ATTACHMENTS. 45
1. Statement of Work. 45
2. Invoice/Financing Request and Contract Financial Reporting Instructions for NIH Cost-Reimbursement Type Contracts, NIH(RC)-4. 45
3. Cumulative Inclusion Enrollment Report. 45
4. Small Business Subcontracting Plan. 45
5. Safety and Health. 45

6. Disclosure of Lobbying Activities, SF-LLL. 45
PART IV - REPRESENTATIONS AND INSTRUCTIONS. 46
SECTION K - REPRESENTATIONS AND CERTIFICATIONS. 46
1. Annual Representations and Certifications. 46

PART I - THE SCHEDULE

SECTION B - SUPPLIES OR SERVICES AND PRICES/COSTS

ARTICLE B.1. BRIEF DESCRIPTION OF SERVICES

The Contractor shall develop EIDD-2801, the 5'-Isoprophl ester prodrug of the broadly active antiviral ribonucleoside analogue EIDD-1931, for the treatment of influenza infections.

ARTICLE B.2. ESTIMATED COST - OPTION

1. The estimated cost of the Base Period of this contract is $1,637,319.
2. If the Government exercises its option pursuant to the OPTION PROVISION Article in SECTION H of this contract, the Government's total estimated contract amount represented by the sum of the estimated cost plus the fixed fee will be increased as follows:

	Total Estimated Cost ($)
Base	$1,637,319
Option One	(b) (4)
Option Two	
Option Three	
Total	

ARTICLE B.3. ADVANCE UNDERSTANDINGS

Other provisions of this contract notwithstanding, approval of the following items within the limits set forth is hereby granted without further authorization from the Contracting Officer.

a. **Confidential Treatment of Sensitive Information**

The Contractor shall guarantee strict confidentiality of the information/data that it is provided by the Government during the performance of the contract. The Government has determined that the information/data that the Contractor will be provided during the performance of the contract is of a sensitive nature.

Disclosure of the information/data, in whole or in part, by the Contractor can only be made after the Contractor receives prior written approval from the Contracting Officer. Whenever the Contractor is uncertain with regard to the proper handling of information/data under the contract, the Contractor shall obtain a written determination from the Contracting Officer.

b. **Contract Number Designation**

On all correspondence submitted under this contract, the Contractor agrees to clearly identify the contract number that appear on the face page of the contract as follows:

Contract No. 75N93019C00058

ARTICLE B.4. PROVISIONS APPLICABLE TO DIRECT COSTS

a. **Items Unallowable Unless Otherwise Provided**

Notwithstanding the clause[s], ALLOWABLE COST AND PAYMENT, [and FIXED FEE,] incorporated in this contract, unless authorized in writing by the Contracting Officer, the costs of the following items or activities shall be unallowable as direct costs:

1. Conferences and Meetings

2. Food for Meals, Light Refreshments, and Beverages

3. Promotional Items *[includes, but is not limited to: clothing and commemorative items such as pens, mugs/cups, folders/folios, lanyards, and conference bags that are sometimes provided to visitors, employees, grantees, or conference attendees.]*

4. Acquisition, by purchase or lease, of any interest in real property;

5. Special rearrangement or alteration of facilities;

6. Purchase or lease of **any** item of general purpose office furniture or office equipment regardless of dollar value. (General purpose equipment is defined as any items of personal property which are usable for purposes other than research, such as office equipment and furnishings, pocket calculators, etc.);

7. Travel to attend general scientific meetings;

8. Foreign travel;

9. Consultant costs;

10. Subcontracts;

11. Patient care costs;

12. Accountable Government Property (defined as non-expendable personal property with an acquisition cost of $1,000 or more) and "sensitive items" (defined as items of personal property (supplies and equipment that are highly desirable and easily converted to personal use), regardless of acquisition value.

13. Printing Costs (as defined in the Government Printing and Binding Regulations).

b. **Travel Costs**

1. The Contractor shall invoice and be reimbursed for all travel costs in accordance with Title 2 of CFR, Part 220-Cost Principles for Educational Institutions (OMB Circular A-21).

SECTION C - DESCRIPTION/SPECIFICATIONS/WORK STATEMENT

ARTICLE C.1. STATEMENT OF WORK

a. Independently and not as an agent of the Government, the Contractor shall furnish all the necessary services, qualified personnel, material, equipment, and facilities, not otherwise provided by the Government as needed to perform the Statement of Work, dated August 1, 2019, set forth in SECTION J-List of Attachments, attached hereto and made a part of this contract.

ARTICLE C.2. REPORTING REQUIREMENTS

All reports required herein shall be submitted in electronic format.

All electronic reports submitted shall be compliant with Section 508 of the Rehabilitation Act of 1973. Additional information about testing documents for Section 508 compliance, including guidance and specific checklists, by application, can be found at: http://www.hhs.gov/web/508/index.html under "Making Files Accessible."

a. **Technical Reports**

In addition to those reports required by the other terms of this contract, the Contractor shall prepare and submit the following reports in the manner stated below and in accordance with the DELIVERIES Article in SECTION F of this contract:

[Note: Beginning May 25, 2008, the Contractor shall include, in any technical progress report submitted, the applicable PubMed Central (PMC) or NIH Manuscript Submission reference number when citing publications that arise from its NIH funded research.]

1. **Monthly Progress Report**

This report shall include a description of the technical activities and results during the reporting period and the activities planned for the ensuing reporting period, and shall include a budget summary for costs incurred during the monthly reporting period for the base period and each option and milestone. The funding level shall be presented in correlation with percent completion of the activities under the base, option and/or milestone.

The first reporting period consists of the first full month of performance plus any fractional part of the initial month. Thereafter, the reporting period shall consist of each calendar month. Monthly Progress Reports shall not be required when the Annual Progress Report is due.

2. **Annual Progress Report**

This report shall include a summation of the technical activities and results for the entire contract period covered, and shall include a description of the technical activities and results during the reporting period, a budget summary for costs incurred during the annual reporting period for the base period and each option and milestone. The funding level shall be presented in correlation with percent completion of the activities under the base, option and/or milestone. An annual report will not be required for the period when the Final Report is due.

3. **Annual Technical Progress Report for Clinical Research Study Populations**

The Contractor shall submit information about the inclusion of women and members of minority groups and their subpopulations (when appropriate) for each study being performed under this contract. In addition, the NIH Policy and Guidelines on the Inclusion of Women and Minorities as Subjects in Clinical Research, Amended, October, 2001 applies: The Guidelines

may be found at the following website: http://grants.nih.gov/grants/funding/women_min/guidelines_amended_10_2001.htm. Include a description of the plans to conduct analyses, as appropriate, by sex/gender and/or racial/ethnic groups in the clinical trial protocol as approved by the IRB, and provide a description of the progress in the conduct of these analyses, as appropriate, in the Annual Progress Report and the Final Report. If the analysis reveals no subset differences, a brief statement to that effect, indicating the subsets analyzed, will suffice. The Government strongly encourages inclusion of the results of subset analysis in all publication submissions. In the final report, the Contractor shall include all final analyses of the data on sex/gender, race and/or ethnicity.

4. **Final Report**
This report is to include a summation of the work performed and the results obtained for the entire contract period of performance. This report shall be in sufficient detail to describe comprehensively the results achieved. The Final Report shall be submitted in accordance with the DELIVERIES Article in SECTION F of this contract. An Annual report will not be required for the period when the Final Report is due.

   The Contractor shall submit, with the Final Report, a summary of salient results (not to exceed 250 words) achieved during the performance of the contract,.

   The Contractor shall provide the Contracting Officer with a copy of the Final Report in **draft** form in accordance with the DELIVERIES Article in SECTION F of this Contract within 30 calendar days prior to the delivery date for the Final Version of the Final Report. The Contracting Officer's Representative (COR) will review the draft report and provide the Contractor with comments within 15 calendar days after receipt. The Final Report shall be corrected by the Contractor, if necessary and the final version delivered as specified in the above paragraph.

5. **Product Development Plan and Work Plan**

   The Contractor shall update the Product Development Plan (PDP) and create a Work Plan to incorporate the progress from the effective date of the contract. The Contractor shall submit an updated PDP for review unless otherwise negotiated with the COR and the Contracting Officer. This updated PDP and Work Plan shall include:

   1. Clearly defined goals, product development stages, and product development activities.
   2. A breakdown of activities by fiscal year and non-severable stages, and applicable decision gates.
   3. Quantitative and qualitative criteria and associated data elements for assessing the scientific merit and feasibility of moving to the next stage of product development.
   4. A detailed timeline for each stage covering the initiation, conduct and completion of product development activities and a task-linked budget (a budget linked to each major activity). A Gantt chart should be provided to outline the proposed activities.
   5. The Work Plan shall include a description of the studies to be performed within each stage of the project. The Contractor shall also be required to submit a revised PDP and associated Work Plan when a change to the approved plans is requested by the COR.
   6. Risk identification, analysis, and mitigation strategies for accomplishing the objectives of thsi contract within the period of performance, particularly with respect to adverse experimental or production results, new scientific findings or regulatory guidance from FDA.

6. **Milestone Completion / Decision Gate Report**

   A Decision Gate Report shall be submitted when the Contractor has completed a stage of product development and has reached a decision point, as defined in the Work Plan for the Implementation

of the Staged Product Development Plan. These reports shall be in sufficient detail to explain comprehensively the results achieved. The description shall also include pertinent data and/or conclusions resulting from the analysis and scientific evaluation of data accumulated to date under the project. Offerors shall propose the timing of these reports to coincide with the decision points specified in their Statement of Work.

7. **Audit Reports**

   Within thirty (30) calendar days of completion of an audit related to conformance to FDA regulations and guidance, including adherence to GLP, GMP, or GCP guidelines, the Contractor shall provide copies of the Audit Report and a plan for addressing areas of nonconformance to FDA regulations and guidance for GLP, GMP, or GCP guidelines as identified in the final Audit Report.

8. **Draft and Final Clinical Trial Protocols**

   NIAID has a responsibility to ensure that mechanisms and procedures are in place to protect the safety of participants in the NIAID-funded clinical trials. Therefore, as described in the NIAID Clinical Terms of Award (https://www.niaid.nih.gov/grants-contracts/niaid-clinical-terms-award), the Contractor shall develop a protocol and all associated documents for each clinical trial and submit drafts for review as well as all final protocols and protocol amendments for approval by DMID. Prior to FDA submission and enrollment, additional reviews and approval periods may be required for changes in the final protocol. Three (3) weeks should be planned for each review period. It is recommended that protocols be submitted using approved DMID templates. The DMID templates and other important information regarding performing human subject research are available at https://www.niaid.nih.gov/grants-contracts/human-subjects.

9. **Draft and Final Clinical Study Report**

   For each clinical study performed with contract support, a Draft Clinical Study Report shall be provided upon completion of the analysis of all data generated in the clinical trial. Following review and approval by DMID, final Clinical Study Reports shall follow the ICH guidelines on Structure and Content of Clinical Study Reports E3 (http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E3/E3_Guideline.pdf).

10. **FDA Correspondence and Meetings**

    Submit for review and approval planned FDA communications as well as any subsequent correspondence and resulting meeting summaries.

11. **Human Subject IRB Annual Report (Form OMB No. 0990-0263)**

    Within thirty (30) calendar days of each anniversary date of the effective contract award, the Contractor shall submit the Human Subject Annual Report.

12. **Clinical Monitoring Plan**

    For each clinical trial performed with contract support, a Clinical Monitoring Plan should be prepared and submitted forty-five (45) calendar days prior to the intended initiation date of the clinical trial.

13. **Clinical Monitoring Reports**

    A copy of each Clinical Monitoring Report shall be provided within thirty (30) calendar days of the completion of the clinical monitoring visit, unless significant GCP violations were discovered in the

clinical monitoring visit. If significant GCP violations were discovered, the Contractor will notify the COR as soon as the Contractor learns about the violation, and the Clinical Monitoring Report shall be provided within fifteen (15) calendar days of the completion of the clinical monitoring visit.

14. **Quality Assurance Reports**

Upon request of the COR, the Contractor shall provide a copy of the QA reports within five (5) calendar days from the COR request.

15. **Safety Oversight Reports**

The Contractor shall provide open (blinded) and closed (unblinded) reports to be reviewed by the safety oversight monitoring board at the intervals specified by the approved clinical protocol. The Contractor shall provide the report at least ten (10) business days prior to each board meeting date in a format mutually agreed upon. The Contractor shall provide shell reports (without any data) at least ten (10) business days prior to the organizational meeting of the safety board.

16. **Samples of Products**

The Contractor shall submit samples of GMP material manufactured with contract funding. The type of material and the amount will be specified in the contract.

17. **Technology Transfer**

Technology Transfer packages shall include complete protocols and critical, assays or procedures developed and/or improved with contract funding.

18. **Institutional Biosafety Approval**

The Contractor shall provide documentation of materials submitted for Institutional Biosafety Committee Review and documentation of approval of experiments.

19. **Annual Contract Review Meeting**

A report of the Post Award Contract Initiation Meeting and Annual Contract Review Meetings shall be prepared by the Contractor and submitted within twenty-one (21) calendar days following the date of the meetings. These reports shall include the slide presentations and all other meeting materials as well as summaries of all discussions.

20. **Teleconference and Meeting Minutes**

Minutes of regular, as well as ad hoc teleconferences and meetings shall be provided by the Contractor within two (2) business days following the date of the teleconference or meeting.

21. **External Advisory Group Meetings**

Reports of all meetings and communications with the External Advisory Group or its individual members shall be documented and submitted to the COR and Contracting Officer. Documentation of such meetings/communications will be provided within twenty-one (21) calendar days and shall include a summary of the discussions and copies of slide presentations.

b. **Other Reports/Deliverables**

1. **Reporting of Financial Conflict of Interest (FCOI)**

   All reports and documentation required by 45 CFR Part 94, Responsible Prospective Contractors including, but not limited to, the New FCOI Report, Annual FCOI Report, Revised FCOI Report, and the Mitigation Report, shall be submitted to the Contracting Officer in Electronic format. Thereafter, reports shall be due in accordance with the regulatory compliance requirements in 45 CFR Part 94.

   45 CFR Part 94 is available at: http://www.ecfr.gov/cgi-bin/text-idx?c=ecfr&SID=0af84ca649a74846f102aaf664da1623&rgn=div5&view=text&node=45:1.0.1.1.51&idno=45. See Part 94.5, Management and reporting of financial conflicts of interest for complete information on reporting requirements.

   (Reference subparagraph g. of the INSTITUTIONAL RESPONSIBILITY REGARDING INVESTIGATOR FINANCIAL CONFLICTS OF INTEREST Article in SECTION H of this contract.)

2. **Section 508 Annual Report**

   The contractor shall submit an annual Section 508 report in accordance with the schedule set forth by the Contracting Officer (CO)/Contracting Officer's Representative (COR). The Section 508 Report Template and Instructions for completing the report are available at: http://www.hhs.gov/web/508/contracting/technology/vendors.html under "Vendor Information and Documents."

REPORTING REQUIREMENTS FOR USE WITH THE ELECTRONIC REPORT DELIVERABLE SUBMISSION (eRDS) SITE

All reports required herein shall be submitted in electronic format. All electronic contract deliverables shall be submitted via the NIAID electronic Report Deliverable Submission (eRDS) Site, available at the following website: https://erds.niaid.nih.gov/. All electronic reports submitted shall be compliant with Section 508 of the Rehabilitation Act of 1973. Additional information about testing documents for Section 508 compliance, including guidance and specific checklists, by application, can be found at http://www.hhs.gov/web/508/index.html under "Making Files Accessible."

ARTICLE C.3. INVENTION REPORTING REQUIREMENT

All reports and documentation required by FAR Clause 52.227-11, Patent Rights-Ownership by the Contractor including, but not limited to, the invention disclosure report, the confirmatory license, and the Government support certification, shall be directed to the Division of Extramural Inventions and Technology Resources (DEITR), OPERA, OER, NIH, 6705 Rockledge Drive, Suite 310, MSC 7980, Bethesda, Maryland 20892-7980 (Telephone: 301-435-1986). In addition, one copy of an annual utilization report, and a copy of the final invention statement, shall be submitted to the Contracting Officer. The final invention statement (see FAR 27.303(b)(2)(ii)) shall be submitted to the Contracting Officer on the expiration date of the contract.

The annual utilization report shall be submitted in accordance with the DELIVERIES Article in SECTION F of this contract. Thereafter, reports shall be due on or before the 30 Calendar day following the reporting period.] The final invention statement (see FAR 27.303(b)(2)(ii)) shall be submitted on the expiration date of the contract. All reports shall be sent to the following address:

Contracting Officer
National Institutes of Health
National Institute of Allergy and Infectious Diseases
Office of Acquisition
5601 Fishers Lane
MSC 9821, Room 3D38
Bethesda, Maryland 20892

If no invention is disclosed or no activity has occurred on a previously disclosed invention during the applicable reporting period, a negative report shall be submitted to the Contracting Officer at the address listed above.

To assist contractors in complying with invention reporting requirements of the clause, the NIH has developed "Interagency Edison," an electronic invention reporting system. Use of Interagency Edison is required as it streamlines the reporting process and greatly reduces paperwork. Access to the system is through a secure interactive Web site to ensure that all information submitted is protected. Interagency Edison and information relating to the capabilities of the system can be obtained from the Web (http://www.iedison.gov), or by contacting the Extramural Inventions and Technology Resources Branch, OPERA, NIH.

SECTION D - PACKAGING, MARKING AND SHIPPING

All deliverables required under this contract shall be packaged, marked and shipped in accordance with Government specifications. At a minimum, all deliverables shall be marked with the contract number and Contractor name. The Contractor shall guarantee that all required materials shall be delivered in immediate usable and acceptable condition.

SECTION E - INSPECTION AND ACCEPTANCE

1. The Contracting Officer or the duly authorized representative will perform inspection and acceptance of materials and services to be provided.
2. For the purpose of this SECTION, the Contracting Officer's Representative is the authorized representative of the Contracting Officer.
3. Inspection and acceptance will be performed at:
   National Institutes of Health
   National Institute of Allergy and Infectious Diseases
   5601 Fishers Lane
   Rockville, Maryland 20852

   Acceptance may be presumed unless otherwise indicated in writing by the Contracting Officer or the duly authorized representative within 30 days of receipt.

d. This contract incorporates the following clause by reference, with the same force and effect as if it were given in full text. Upon request, the Contracting Officer will make its full text available.

   FAR Clause **52.246-9, Inspection of Research and Development (Short Form)** (April 1984).

SECTION F - DELIVERIES OR PERFORMANCE

ARTICLE F.1. COMPLETION DATE

1. The completion date of this contract is March 8, 2020.
2. If the Government exercises its option(s) pursuant to the OPTION PROVISION Article in Section H of this contract, the period of performance will be increased as listed below:

Option	Option Period
Option One	**14 months from the Effective Date of the Option**
Option Two	**6 months from the Effective Date of the Option**
Option Three	**12 months from the Effective Date of the Option**

ARTICLE F.2. DELIVERIES

Satisfactory performance of the final contract shall be deemed to occur upon performance of the work described in the Statement of Work Article in SECTION C of this contract and upon delivery and acceptance by the Contracting Officer, or the duly authorized representative, of the following items in accordance with the stated delivery schedule:

1. The items specified below as described in the REPORTING REQUIREMENTS Article in SECTION C of this contract will be required to be delivered F.o.b. Destination as set forth in FAR 52.247-35, F.o.b. DESTINATION, WITHIN CONSIGNEES PREMISES (APRIL 1984), and in accordance with and by the date(s) specified below [and any specifications stated in SECTION D, PACKAGING, MARKING AND SHIPPING, of this contract]:

Item	Description	Quantity	Delivery Schedule
(1)	Monthly Progress Reports	One electronic copy via eRDS	On or before 15 calendar days following each reporting period.
(2)	Annual Progress Reports	One electronic copy via eRDS	On or before 30 calendar days following each reporting period.
(3)	Annual Technical Progress Report for Clinical Research Study Populations	One electronic copy via eRDS	On or before 30 calendar days following each reporting period. Final report due on or before contract completion date.
(4)	Final Report	One electronic copy via eRDS	Draft Final Report due on or before 30 calendar days prior to contract completion date. Final Report due on or before contract completion date.
(5)	Product Development Plan and Work Plan	One electronic copy via eRDS	Within 30 calendar days of the effective date of the contract and prior to initiation of product development activities.
(6)	Milestone Completion / Decision Gate Report	One electronic copy via eRDS	Within 15 calendar days after a stage of the product development has been completed and reached a decision point as defined in the Work Plan.
(7)	Audit Report	One electronic copy via eRDS	Within 30 calendar days of completion of an audit.
(8)	Final Clinical Trial Protocols and Protocol Amendments	One electronic copy via eRDS	Within 15 calendar days following approval of final protocol and protocol amendments.
(9)	Final Clinical Study Report	One electronic copy via eRDS	On or before contract completion date.
(10)	FDA Correspondence and Meetings Summaries	One electronic copy via eRDS	Within 5 calendar days of receiving correspondence from or holding meeting with the FDA.

Item	Description	Quantity	Delivery Schedule
(11)	Human Subject IRB Annual Report	One electronic copy via eRDS	Within 30 calendar days following each reporting period.
(12)	Clinical Monitoring Plan	One electronic copy via eRDS	Within 45 calendar days prior to initiation date of the clinical trial
(13)	Clinical Monitoring Report	One electronic copy via eRDS	Within 30 calendar days of the completion of clinical monitoring visit.
(14)	Quality Assurance Report	One electronic copy via eRDS	Within 5 calendar days from COR's request.
(15)	Safety Oversight Report	One electronic copy via eRDS	Within 10 calendar days prior to each board meeting date.
(16)	Sample of Products	As directed by COR	On or before contract completion date.
(17)	Technology Transfer	One electronic copy via eRDS	On or before contract completion date.
(18)	Institutional Biosafety Approval	One electronic copy via eRDS	Within 10 calendar days after approval from Institutional Biosafety Committee Review.
(19)	Contract Initiation Meeting and Annual Contract Review Meeting Reports	One electronic copy via eRDS	Within 21 calendar days following the date of the meetings.
(20)	Teleconference and Meeting Minutes	One electronic copy via eRDS	Within 2 business days following the date of the teleconference or meeting.
(21)	External Advisory Group Meetings Reports	One electronic copy via eRDS	Within 21 calendar days following the date of the meeting.

2. The above items shall be addressed and delivered to:

Addressee	Deliverable Item No	Quantity
Contracting Officer and COR	Items 1-15 and 17-21	One electronic copy via eRDS
COR	Item 16	As directed by COR

ARTICLE F.3. CLAUSES INCORPORATED BY REFERENCE, FAR 52.252-2 (FEBRUARY 1998)

This contract incorporates the following clause(s) by reference, with the same force and effect as if it were given in full text. Upon request, the Contracting Officer will make its full text available. Also, the full text of a clause may be accessed electronically at this address: https://www.acquisition.gov/?q=browsefar.

FEDERAL ACQUISITION REGULATION (48 CFR CHAPTER 1) CLAUSE:

52.242-15, Stop Work Order (August 1989)

Alternate I (April 1984) is applicable to this contract.

SECTION G - CONTRACT ADMINISTRATION DATA

ARTICLE G.1. CONTRACTING OFFICER'S REPRESENTATIVE (COR)

The following Contracting Officer's Representative (COR) will represent the Government for the purpose of this contract:

Helen Schiltz, Ph.D.
Anita Sheoran, Ph.D. - Alternate COR

The COR is responsible for: (1) monitoring the Contractor's technical progress, including the surveillance and assessment of performance and recommending to the Contracting Officer changes in requirements; (2) interpreting the statement of work and any other technical performance requirements; (3) performing technical evaluation as required; (4) performing technical inspections and acceptances required by this contract; and (5) assisting in the resolution of technical problems encountered during performance.

The alternate COR is responsible for carrying out the duties of the COR only in the event that the COR can no longer perform her duties as assigned.

The Contracting Officer is the only person with authority to act as agent of the Government under this contract. Only the Contracting Officer has authority to: (1) direct or negotiate any changes in the statement of work; (2) modify or extend the period of performance; (3) change the delivery schedule; (4) authorize reimbursement to the Contractor for any costs incurred during the performance of this contract; (5) otherwise change any terms and conditions of this contract; or (6) sign written licensing agreements. Any signed agreement shall be incorporated by reference in Section K of the contract

The Government may unilaterally change its COR designation.

ARTICLE G.2. KEY PERSONNEL, HHSAR 352.237-75 (December 2015)

The key personnel specified in this contract are considered to be essential to work performance. At least 30 days prior to the contractor voluntarily diverting any of the specified individuals to other programs or contracts the Contractor shall notify the Contracting Officer and shall submit a justification for the diversion or replacement and a request to replace the individual. The request must identify the proposed replacement and provide an explanation of how the replacement's skills, experience, and credentials meet or exceed the requirements of the contract (including, when applicable, Human Subjects Testing requirements). If the employee of the contractor is terminated for cause or separates from the contractor voluntarily with less than thirty days notice, the Contractor shall provide the maximum notice practicable under the circumstances. The Contractor shall not divert, replace, or announce any such change to key personnel without the written consent of the Contracting Officer. The contract will be modified to add or delete key personnel as necessary to reflect the agreement of the parties.

(End of Clause)

The following individual is considered to be essential to the work being performed hereunder:

Name	Title
(b) (6), (b) (3) (A)	Principal Investigator

ARTICLE G.3. INVOICE SUBMISSION/CONTRACT FINANCING REQUEST AND CONTRACT FINANCIAL REPORT

a. Invoice Submission/Contract Financing Request and Contract Financial Reporting, NIH(RC)-4 for NIH Cost-Reimbursement Type Contracts are attached and made part of this contract. The Contractor shall follow the

attached instructions and submission procedures specified below to meet the requirements of a "proper invoice" pursuant to FAR Subpart 32.9, Prompt Payment.

1. Payment requests shall be submitted to the offices identified below. **Do not submit supporting documentation (e.g., receipts, time sheets, vendor invoices, etc.) with your payment request unless specified elsewhere in the contract or requested by the Contracting Officer.**

   a. The original invoice shall be submitted to the following **designated billing office**:

      National Institutes of Health
      Office of Financial Management
      Commercial Accounts
      2115 East Jefferson Street, Room 4B-432, MSC 8500
      Bethesda, MD 20892-8500

   b. One copy of the invoice shall be submitted to the following **approving official**:

      Contracting Officer
      Office of Acquisitions
      DEA, NIAID, NIH
      5601 Fishers Lane Room 3D38
      Bethesda MSC 9821
      Maryland 20892- 9821

      E-mail: teosl@niaid.nih.gov

      The Contractor shall submit an electronic copy of the payment request to the approving official instead of a paper copy. The payment request shall be transmitted as an attachment via e-mail to the address listed above in one of the following formats: MSWord, MS Excel, or Adobe Portable Document Format (PDF). Only one payment request shall be submitted per e-mail and the subject line of the e-mail shall include the Contractor's name, contract number, and unique invoice number. ***[Note: The original payment request must still be submitted in hard copy and mailed to the designated billing office to meet the requirements of a "proper invoice."]***

      Central Point of Distribution: NIAID_MIDARCB_Invoices

      The Contractor shall include the Central Point of Distribution on the invoice.

2. In addition to the requirements specified in FAR 32.905 for a proper invoice, the Contractor shall include the following information on the face page of all payment requests:

   a. Name of the Office of Acquisitions. The Office of Acquisitions for this contract is National Institute of Allergy and Infectious Diseases .

   b. Federal Taxpayer Identification Number (TIN). If the Contractor does not have a valid TIN, it shall identify the Vendor Identification Number (VIN) on the payment request. The VIN is the number that appears after the Contractor's name on the face page of the contract. *[Note: A VIN is assigned to new contracts awarded on or after June 4, 2007, and any existing contract modified to include the VIN number.]* If the Contractor has neither a TIN, DUNS, or VIN, contact the Contracting Officer.

   c. DUNS or DUNS+4 Number. The DUNS number must identify the Contractor's name and address exactly as stated in the contract and as registered in the Central Contractor Registration (CCR) database. If the Contractor does not have a valid DUNS number, it shall identify the

Vendor Identification Number (VIN) on the payment request. The VIN is the number that appears after the Contractor's name on the face page of the contract. *[Note: A VIN is assigned to new contracts awarded on or after June 4, 2007, and any existing contract modified to include the VIN number.]* If the Contractor has neither a TIN, DUNS, or VIN, contact the Contracting Officer.

d. Invoice Matching Option. This contract requires a two-way match.

e. Unique Invoice Number. Each payment request must be identified by a unique invoice number, which can only be used one time regardless of the number of contracts or orders held by an organization.

f. The Contract Title is:

The development of EIDD-2801, the 5'-Isoprophy ester prodrug of the broadly active antiviral ribonucleoside analogue EIDD-1931, for the treatment of influenza infections.

b. Inquiries regarding payment of invoices shall be directed to the designated billing office, (301) 496-6452.

c. The Contractor shall include the following certification on every invoice for reimbursable costs incurred with Fiscal Year funds subject to HHSAR Clause 352.231-70, Salary Rate Limitation in SECTION I of this contract. For billing purposes, certified invoices are required for the billing period during which the applicable Fiscal Year funds were initially charged through the final billing period utilizing the applicable Fiscal Year funds:

"I hereby certify that the salaries charged in this invoice are in compliance with HHSAR Clause 352.231-70, Salary Rate Limitation in SECTION I of the above referenced contract."

ARTICLE G.4. PROVIDING ACCELERATED PAYMENT TO SMALL BUSINESS SUBCONTRACTORS, FAR 52.232-40 (December 2013)

a. Upon receipt of accelerated payments from the Government, the Contractor shall make accelerated payments to its small business subcontractors under this contract, to the maximum extent practicable and prior to when such payment is otherwise required under the applicable contract or subcontract, after receipt of a proper invoice and all other required documentation from the small business subcontractor.

b. The acceleration of payments under this clause does not provide any new rights under the prompt Payment Act.

c. Include the substance of this clause, include this paragraph c, in all subcontracts with small business concerns, including subcontracts with small business concerns for the acquisition of commercial items.

(End of Clause)

ARTICLE G.5. INDIRECT COST RATES

In accordance with Federal Acquisition Regulation (FAR) (48 CFR Chapter 1) Clause 52.216-7 (d)(2), Allowable Cost and Payment incorporated by reference in this contract in PART II, SECTION I, the cognizant Contracting Officer representative responsible for negotiating provisional and/or final indirect cost rates is identified as follows:

Director, Division of Financial Advisory Services
Office of Acquisition Management and Policy
National Institutes of Health
6011 EXECUTIVE BLVD, ROOM 549C, MSC-7663
BETHESDA MD 20892-7663

These rates are hereby incorporated without further action of the Contracting Officer.

ARTICLE G.6. POST AWARD EVALUATION OF CONTRACTOR PERFORMANCE

1. Contractor Performance Evaluations

   Interim and Final evaluations of Contractor performance will be prepared on this contract in accordance with FAR Subpart 42.15. The Final performance evaluation will be prepared at the time of completion of work. In addition to the Final evaluation, Interim evaluation(s) will be prepared annually on the anniversary date of the contract's effective date.

   Interim and Final evaluations will be provided to the Contractor as soon as practicable after completion of the evaluation. The Contractor will be permitted sixty days to review the document and to submit additional information or a rebutting statement. If agreement cannot be reached between the parties, the matter will be referred to an individual one level above the Contracting Officer, whose decision will be final.

   Copies of the evaluations, Contractor responses, and review comments, if any, will be retained as part of the contract file, and may be used to support future award decisions.

2. Electronic Access to Contractor Performance Evaluations

   Contractors may access evaluations through a secure Web site for review and comment at the following address:

   http://www.cpars.gov

SECTION H - SPECIAL CONTRACT REQUIREMENTS

ARTICLE H.1. PROTECTION OF HUMAN SUBJECTS, HHSAR 352.270-4(b) (December 2015)

1. The Contractor agrees that the rights and welfare of human subjects involved in research under this contract shall be protected in accordance with 45 CFR part 46 and with the Contractor's current Federal-wide Assurance (FWA) on file with the Office for Human Research Protections (OHRP), Department of Health and Human Services. The Contractor further agrees to provide certification at least annually that the Institutional Review Board has reviewed and approved the procedures, which involve human subjects in accordance with 45 CFR part 46 and the Assurance of Compliance.

2. The Contractor shall bear full responsibility for the performance of all work and services involving the use of human subjects under this contract and shall ensure that work is conducted in a proper manner and as safely as is feasible. The parties hereto agree that the Contractor retains the right to control and direct the performance of all work under this contract. Nothing in this contract shall create an agency or employee relationship between the Government and the Contractor, or any subcontractor, agent or employee of the Contractor, or any other person, organization, institution, or group of any kind whatsoever. The Contractor agrees that it has entered into this contract and will discharge its obligations, duties, and undertakings and the work pursuant thereto, whether requiring professional judgment or otherwise, as an independent Contractor without creating liability on the part of the Government for the acts of the Contractor or its employees.

3. Contractors involving other agencies or institutions in activities considered to be engaged in research involving human subjects must ensure that such other agencies or institutions obtain their own FWA if they are routinely engaged in research involving human subjects or ensure that such agencies or institutions are covered by the Contractors' FWA via designation as agents of the institution or via individual investigator agreements (see OHRP Website at: http://www.hhs.gov/ohrp/policy/guidanceonalternativetofwa.pdf).

4. If at any time during the performance of this contract the Contractor is not in compliance with any of the requirements and or standards stated in paragraphs (a) and (b) above, the Contracting Officer may immediately suspend, in whole or in part, work and further payments under this contract until the Contractor corrects the noncompliance. The Contracting Officer may communicate the notice of suspension by telephone with confirmation in writing. If the Contractor fails to complete corrective action within the period of time designated in the Contracting Officer's written notice of suspension, the Contracting Officer may, after consultation with OHRP, terminate this contract in whole or in part.

5. (End of clause)

ARTICLE H.2. HUMAN SUBJECTS

Research involving human subjects shall not be conducted under this contract until the protocol developed in Phase I has been approved by NIAID (INSERT IC), written notice of such approval has been provided by the Contracting Officer, and the Contractor has provided to the Contracting Officer a properly completed "Protection of Human Subjects Assurance Identification/IRB Certification/Declaration of Exemption", Form OMB No. 0990-0263 (formerly Optional Form 310) certifying IRB review and approval of the protocol. The human subject certification can be met by submission of the Contractor's self designated form, **provided** that it contains the information required by the "Protection of Human Subjects Assurance Identification/IRB Certification/Declaration of Exemption", Form OMB No. 0990-0263 (formerly Optional Form 310).

When research involving Human Subjects will take place at collaborating sites or other performance sites, the Contractor shall obtain, and keep on file, a properly completed "Protection of Human Subjects Assurance Identification/IRB Certification/Declaration of Exemption", Form OMB No. 0990-0263 (formerly Optional Form 310) certifying IRB review and approval of the research.

ARTICLE H.3. RESTRICTION ON USE OF HUMAN SUBJECTS, HHSAR 352.270-6 (December 2015)

Pursuant to 45 CFR part 46, Protection of Human Research Subjects, the Contractor shall not expend funds under this award for research involving human subjects or engage in any human subjects research activity prior to the Contracting Officer's receipt of a certification that the research has been reviewed and approved by the Institutional Review Board (IRB) registered with OHRP. This restriction applies to all collaborating sites, whether domestic or foreign, and subcontractors. The Contractor must ensure compliance by collaborators and subcontractors.

(End of clause)

ARTICLE H.4. REQUIRED EDUCATION IN THE PROTECTION OF HUMAN RESEARCH PARTICIPANTS

NIH policy requires education on the protection of human subject participants for all investigators receiving NIH contract awards for research involving human subjects. For a complete description of the NIH Policy announcement on required education in the protection of human subject participants, the Contractor should access the NIH Guide for Grants and Contracts Announcement dated June 5, 2000 at the following website:

http://grants.nih.gov/grants/guide/notice-files/NOT-OD-00-039.html.

The information below is a summary of the NIH Policy Announcement:

The Contractor shall maintain the following information: (1) a list of the names and titles of the principal investigator and any other individuals working under the contract who are responsible for the design and/or conduct of the research; (2) the title of the education program(s) in the protection of human subjects that has been completed for each named personnel and; (3) a one sentence description of the educational program(s) listed in (2) above. This requirement extends to investigators and all individuals responsible for the design and/or conduct of the research who are working as subcontractors or consultants under the contract.

Prior to any substitution of the Principal Investigator or any other individuals responsible for the design and/or conduct of the research under the contract, the Contractor shall provide the following written information to the Contracting Officer: the title of the education program and a one sentence description of the program that has been completed by the replacement.

ARTICLE H.5. DATA AND SAFETY MONITORING IN CLINICAL TRIALS

The Contractor is directed to the full text of the NIH Policy regarding Data and Safety Monitoring and Reporting of Adverse Events, which may be found at the following web sites:

http://grants.nih.gov/grants/guide/notice-files/not98-084.html
http://grants.nih.gov/grants/guide/notice-files/not99-107.html
http://grants.nih.gov/grants/guide/notice-files/NOT-OD-00-038.html

The Contractor must comply with the NIH Policy cited in these NIH Announcements and any other data and safety monitoring requirements found elsewhere in this contract.

Data and Safety Monitoring shall be performed in accordance with the approved Data and Safety Monitoring Plan.

The Data and Safety Monitoring Plan shall be established and approved prior to beginning the conduct of the clinical trial.

ARTICLE H.6. GOOD CLINICAL PRACTICE TRAINING FOR NIH AWARDEES INVOLVED IN NIH-FUNDED CLINICAL TRIALS

All NIH-funded investigators and staff who are involved in the conduct, oversight, or management of clinical trials should be trained in Good Clinical Practice (GCP), consistent with principles of the International Conference on Harmonisation (ICH) E6 (R2). GCP training may be achieved through a class or course, academic training program, or certification from a recognized clinical research professional organization. GCP training should be refreshed at least every three years to remain current with regulations, standards and guidelines. The Contractor shall provide completion of training documentation to the Contracting Officer's Representative (COR).

Investigator: The individual responsible for the conduct of the clinical trial at a trial site. If a clinical trial is conducted by a team of individuals at a trial site, the investigator is the responsible leader of the team and may be called the principal investigator.

Clinical Trial Staff: Individuals, identified by the investigator, who are responsible for study coordination, data collection and data management. Clinical trial staff may also be called the research coordinator, study coordinator, research nurse, study nurse or sub-investigator.

ARTICLE H.7. CLINICAL TRIAL REGISTRATION AND RESULTS INFORMATION SUBMISSION

The Contractor conducting clinical trials, funded wholly or partially through the NIH extramural and intramural programs, shall ensure that its NIH-funded clinical trials are registered at, and summary results information is submitted to, www.clinicaltrials.gov for public posting. See NIH Guide Notice NOT-OD-16-149 dated September 16, 2016.
All NIH-funded clinical trials shall be registered and results information submitted to www.clinicaltrials.gov regardless of study phase, type of intervention, or whether they are subject to the regulation 42 CFR Part 11. Clinical trials subject to the regulation are called "applicable clinical trials."
The Contractor must submit a plan with its proposal to meet the regulatory requirements of the dissemination of information of NIH-funded Clinical Trials. The Contractor and investigators are required to comply with all terms and conditions of award, including following their acceptable plan for the dissemination of NIH-funded clinical trial information.
The Contractor must register all NIH-funded clinical trials in www.clinicaltrials.gov not later than 21 calendar days after the enrollment of the first participant. Results information from those trials must be submitted not later than one year after the trial's primary completion date. Submission of results information can be delayed in certain circumstances for up to two additional years for trials of products regulated by the FDA that are unapproved, unlicensed, or uncleared or for trials of products for which approval, licensure, or clearance of a new use is being sought. The Contractor shall

include the trial registration number (NCT number) in the Technical Progress Report covering the period in which registration occurred, and as a standalone notification to the Contracting Officer within ten (10) calendar days of the registration. Each NIH-funded clinical trial must have only one entry in ClinicalTrials.gov that contains its registration and results information
The Contractor shall include a specific statement in all informed consent documents relating to posting of clinical trials information to www.clinicaltrials.gov. The responsibilities of the Contractor will fall within one of the following three categories:

1. If the NIH-funded clinical trial is an applicable clinical trial under the regulation and the Contractor is the responsible party, the Contractor will ensure that all regulatory requirements are met.
2. If the NIH-funded clinical trial is an applicable clinical trial under the regulation but the Contractor is not the responsible party, the Contractor will coordinate with the responsible party to ensure that all regulatory requirements are met.
3. If the NIH-funded clinical trial is not an applicable clinical trial under the regulation, the Contractor will be responsible for carrying out the tasks and meeting the timelines described in regulation. Such tasks include registering the clinical trial in ClinicalTrials.gov and submitting results information to ClinicalTrials.gov.

Failure to comply with the terms and conditions of the award may provide a basis for enforcement actions. Identifying clinical trial record as non-compliant in ClinicalTrials.gov may lead to termination, consistent with 45 CFR 75.371 and/or other authorities, as appropriate. If the NIH-funded clinical trial is also an applicable clinical trial, non-compliance with the requirements specified in 42 USC 282(j) and 42 CFR Part 11 may also lead to the actions described in 42 CFR 11.66.

The Contracting Officer may take one or more of the following enforcement actions, if the Contractor fails to provide evidence of compliance within 30 days.

- Temporary withhold payments pending correction of the deficiency;
- Disallow all or part of the cost of the activity or action not in compliance;
- Wholly or partly suspend or terminate the contract award;
- Initiate suspension or debarment proceedings as authorized under 2 CFR part 180 and HHS awarding regulations at 2 CFR part 376;
- Withhold further awards for the project and program;
- Take other remedies that may be legally available.

ARTICLE H.8. CLINICAL TRIAL REGISTRATION AND RESULTS INFORMATION SUBMISSION PLAN

The special terms and conditions in the Contract Award that include a clinical trial:

1. The clinical trial(s) supported by this award is subject to the plan submitted to NIH and the NIH policy on Dissemination of NIH-Funded Clinical Trial Information. The plan must state that the clinical trial(s) funded by this award will be registered in ClinicalTrials.gov not later than 21 calendar days after enrollment of the first participant. The plan also must state that primary summary results shall be reported in ClinicalTrials.gov, including adverse event information, not later than one year after the primary completion date of the trial. The reporting of summary results is required by this term of award.
2. This award is subject to reporting requirements with each submission of the annual report. Contractor shall agree to the following annual certification. By affirming this annual certification:

The Contractor hereby certifies that all investigators conducting NIH-funded clinical trials under the NIH contract number 75N93019C00058 are in compliance with the Contractor's plan addressing compliance with the NIH policy on Dissemination of NIH-Funded Clinical Trial Information. Any clinical trial funded wholly or partially under this award has been registered in ClinicalTrials.gov or will be registered not later than 21 calendar days after enrollment of the first participant. Primary summary results have been submitted to ClinicalTrials.gov or will be submitted not later than one year after the primary completion date of the trial.

ARTICLE H.9. CERTIFICATE OF CONFIDENTIALITY

Section 2012 of the 21st Century Cures Act, enacted December 13, 2016, enacts new provisions governing the authority of the Secretary of Health and Human Services (Secretary) to protect the privacy of individuals who are the subjects of research, including significant amendments to the previous statutory authority for such protections, under subsection 301(d) of the Public Health Service Act.
Effective October 1, 2017, all research that was commenced or ongoing on or after December 13, 2016 and is within the scope of the NIH Policy for Issuing Certificate of Confidentiality (CoC) NOT-OD-17-109, the Contractor shall protect the privacy of individuals who are subjects of such research in accordance with subsection 301(d) of the Public Health Service Act as a term and condition of the contract. The certificate will not be issued as a separate document.

NIH considers research in which identifiable, sensitive information is collected or used, to include:

- Human subjects research as defined in the Federal Policy for the Protection of Human Subjects (45 CFR 46), including exempt research (except for human subjects' research that is determined to be exempt from all or some of the requirements of 45 CFR 46) if the information obtained is recorded in such a manner that human subjects cannot be identified or the identity of the human subjects cannot readily be ascertained, directly or through identifiers linked to the subjects;
- Research involving the collection or use of biospecimens that are identifiable to an individual or for which there is at least a very small risk that some combination of the biospecimen, a request for the biospecimen, and other available data sources could be used to deduce the identity of an individual;
- Research that involves the generation of individual level, human genomic data from biospecimens, or the use of such data, regardless of whether the data is recorded in such a manner that human subjects can be identified or the identity of the human subjects can readily be ascertained as defined in the Federal Policy for the Protection of Human Subjects (45 CFR 46); or
- Any other research that involves information about an individual for which there is at least a very small risk, as determined by current scientific practices or statistical methods, that some combination of the information, a request for the information, and other available data sources could be used to deduce the identity of an individual, as defined in subsection 301(d) of the Public Health Service Act.

The Contractor shall not:

- Disclose or provide, in any Federal, State, or local civil, criminal, administrative, legislative, or other proceeding, the name of such individual or any such information, document, or biospecimen that contains identifiable, sensitive information about the individual and that was created or compiled for purposes of the research, unless such disclosure or use is made with the consent of the individual to whom the information, document, or biospecimen pertains; or
- Disclose or provide to any other person not connected with the research the name of such an individual or any information, document, or biospecimen that contains identifiable, sensitive information about such an individual and that was created or compiled for purposes of the research.

The Contractor is permitted to disclose only in below circumstances. The Contractor shall notify the CO minimum ten (10) calendar days prior to disclosure.

- Required by Federal, State, or local laws (e.g., as required by the Federal Food, Drug, and Cosmetic Act, or state laws requiring the reporting of communicable diseases to State and local health departments),

excluding instances of disclosure in any Federal, State, or local civil, criminal, administrative, legislative, or other proceeding;

- Necessary for the medical treatment of the individual to whom the information, document, or biospecimen pertains and made with the consent of such individual;
- Made with the consent of the individual to whom the information, document, or biospecimen pertains; or
- Made for the purposes of other scientific research that is in compliance with applicable Federal regulations governing the protection of human subjects in research.

In accordance with 45 CFR Part 75.303(a), the contractor shall maintain effective internal controls (e.g., policies and procedures) that provide reasonable assurance that the award is managed in compliance with Federal Statutes and regulations.

The recipient of CoCs shall ensure that any company/institution/individual not funded by NIH who receives a copy of identifiable, sensitive information protected by a Certificate is subject to the requirements of subsection 301(d) of the Public Health Service Act. The Contractor shall ensure that Subcontractors who receive funds to carry out part of the Federal award are subject to subsection 301(d) of the Public Health Service Act and the NIH Policy for Issuing CoC.

ARTICLE H.10. SINGLE INSTITUTIONAL REVIEW BOARD (sIRB)

For Institutional Review Board (IRB), the Contractor shall use the single Institutional Review Board (sIRB) of record for multi-site research. All domestic sites participating in multi-site studies involving a non-exempt human subjects research funded wholly or partially by the National Institutes of Health (NIH) shall use a sIRB to conduct the ethical review required by the Department of Health and Human Services regulations for the Protection of Human Subjects at 45 CFR Part 46 and the NIH Policy on the Use of Single Institutional Review Board for Multi-Site Research. Any IRB serving as the sIRB of record for NIH funded research shall be registered with the HHS Office for Human Research Protections (OHRP) and shall have membership sufficient to adequately review the proposed study.

The Contractor shall provide to the Contracting Officer a properly completed "Protection of Human Subjects Assurance Identification/IRB Certification/Declaration of Exemption", Form OMB No. 0990-0263 certifying IRB review and approval of the research that encompasses all sites of performance.

This paragraph applies only if the Government provided a sIRB through a separate entity as stated in section- C. When the Government provided sIRB through a separate entity, the Contractor agrees to use of the sIRB. The Contractor shall provide to the Contracting Officer sIRB information and data in a timely manner as necessary to meet the policy and/or regulatory requirements of the Protection of Human Subjects at 45 CFR Part 46.

Exceptions to the NIH Single IRB Policy

The Contractor may request an exception in the following instances:

1. Sites for which Federal, state, or tribal laws, regulations or policies require local IRB review (policy-based exceptions);
2. *Other exceptions*, to be determined by NIH if there is a compelling justification; and
3. Time Limited Exception: ancillary studies to ongoing research without a sIRB- new multi-site non-exempt human subjects' ancillary studies, that would otherwise be expected to comply with the sIRB policy, but are associated with the ongoing multi-site parent studies, will not be required to use a sIRB of record until the parent study is expected to comply with the sIRB policy.

Policy-based exceptions and time limited exceptions are automatically granted when identified in the sIRB Plan.

Other exceptions must be reviewed by NIH sIRB Exceptions Review Committee (ERC) and are expected to be granted rarely. *Other exceptions* when Offeror believes that one or more research sites should be exempt from use of the single IRB of record to conduct local IRB review based on compelling justification-

a. Offerors should request an exception in the sIRB plan attachment within the contract proposal (section 3.2 in the Study Record: PHS Human Subjects and Clinical Trials Information form).

b. Offerors must include the name of the site(s) for which an IRB other than the sIRB of record is proposed to review the study for the sites(s).

c. Offerors must substantiate their exception request with sufficient information that demonstrates a compelling justification for *other exceptions* to the sIRB policy. The rationale should include why the sIRB of record cannot serve as the reviewing IRB for the site(s), and why the local IRB is uniquely qualified to be the reviewing IRB for the specific site(s).

- For instance, the justification may consider ethical or human subjects protections issues, population needs, or other compelling reasons that IRB review for the site(s) cannot be provided by the single IRB of record.

d. Note that the proposed budget in the proposal must reflect all necessary sIRB costs without an approved *other exception*. The Offerors should not assume that an *other exception* will be granted when considering what sIRB costs to include in the budget.

Post-Award Exception Requests

For any post-award changes that necessitate an exception request, such as the addition of a new domestic site that may be unable to use the sIRB Contractor shall contact their Contracting Officer (CO). For policy-based exceptions, the Contractor shall provide the appropriate citation to verify the requirement for local IRB review for the newly added site(s) to the CO. For *other exceptions*, the Contractor shall provide compelling justification to the CO to be reviewed by the NIH Exceptions Review Committee (ERC) (see **Steps to Request an Other Exception to the sIRB Policy** above). For time limited exceptions, Contractor shall provide the parent contract number to the CO. For time limited exceptions, Contractor shall provide the parent contract number to the CO.

Notice of Approval or Disapproval of *Other Exception* Requests

The sIRB exception requests will be considered after peer review for proposals in the competitive range. The decision of NIH ERC is final. Offerors will be notified of the final decision by their CO prior to award. Approved exceptions will be incorporated as a term and condition in the contract award. Also, any exception requests submitted after award must be submitted to the CO and reviewed by the NIH ERC. No further revisions of the exception request will be accepted.

The award budget may need to be adjusted if an exception is granted.

ARTICLE H.11. PLAN FOR SINGLE INSTITUTIONAL REVIEW BOARD (sIRB)

For this multi-site study, the Contractor agrees to adhere to the NIH sIRB policy, and the IRB shall serve as the single IRB of record. All participating sites have agreed to rely on the IRB, and a written authorization/reliance agreement shall be developed. Any additional sites added after contract award shall also agree to rely on this study's single IRB of record. Communication plans for interactions between the sIRB and participating sites shall be described in the authorization/reliance agreement. All participating sites shall, prior to initiating the study, sign the authorization/reliance agreement that shall clarify the roles and responsibilities of the sIRB and participating sites. The Contractor shall maintain records of the authorization/reliance agreements, including the communication plans. The approved sIRB plan will be incorporated as a term and condition of the award. Any updates/changes to the plan

shall be provided to the Contracting Officer's Representative with a copy submitted to the Contracting Officer within 30 calendar days.

Exceptions to the Single IRB Plan

The Contractor may request an exception to the sIRB plan under the following instances:

- Sites for which federal, state, or tribal laws, regulations or policies require local IRB review (policy-based exceptions)

*Review by a single IRB of record will not be possible for (**sites)** because of federal/state/tribal law, regulation, or policy (**provide specific citation(s))***

- *Other exceptions*, to be determined by NIH if there is a compelling justification

*Review by a single IRB of record will not be possible for (**this contractor)** because of (**provide compelling justification and rationale why local IRB is uniquely qualified to be the reviewing IRB for the specific site(s**)).*

- Time Limited Exceptions: New multi-site non-exempt human subjects' ancillary studies, that would otherwise be expected to comply with the sIRB policy, but are associated with the ongoing multi-site parent studies, will not be required to use the sIRB of record until the parent study is expected to comply with the sIRB policy.

*Review by a single IRB of record will not be possible for (**sites)** because of ongoing multi-site parent study (**provide parent contract number)**.*

ARTICLE H.12. INCLUSION OF WOMEN AND MINORITIES IN RESEARCH INVOLVING HUMAN SUBJECTS

NIH-conducted and supported clinical research must conform to the NIH Policy and Guidelines on the Inclusion of Women and Minorities as Subjects in Clinical Research in accord with Public Health Service Act sec. 4928 U.S.C. sec 289a-2. The policy requires that women and members of minority groups and their subpopulations must be included in all NIH-conducted or supported clinical research projects involving human subjects, unless a clear and compelling rationale and justification establishes to the satisfaction of the relevant NIH Institute/Center (IC) Director that inclusion is inappropriate with respect to the health of the subjects or the purpose of the research. The Director, NIH, may determine that exclusion under other circumstances is acceptable, upon the recommendation of an IC Director, based on a compelling rationale and justification. Cost is not an acceptable reason for exclusion except when the study would duplicate data from other sources. Women of childbearing potential should not be routinely excluded from participation in clinical research.

All investigators proposing research involving human subjects should read the UPDATED "NIH Policy and Guidelines on the Inclusion of Women and Minorities as Subjects in Clinical Research, Amended November 2017," published in the NIH Guide for Grants and Contracts on October 9, 2001 at the following web site:

http://grants.nih.gov/grants/funding/women_min/guidelines_amended_10_2001.htm.

The Contractor must submit the results of valid analyses by sex/gender and race/ethnicity to Clinicaltrials.gov for all NIH-conducted or supported applicable NIH-defined Phase III clinical trials. This requirement does not apply to NIH-defined Phase III trials not considered to applicable clinical trials under 42 CFR Part 11. The Contractor must report applicable NIH-defined Phase III clinical trials involving research subjects of all ages, including foreign awards and domestic awards with a foreign component. The Contractor must specify outcomes on sex/gender and race/ethnicity, as required based on prior evidence, and as explained in the NIH Policy and Guidelines on the Inclusion of Women and Minorities as Subjects in Clinical Research.

Note: Applicable clinical trials are required to be registered in ClinicalTrials.gov not later than 21 calendar days after the enrollment of the first participant. Results information, including the results of the valid analyses by sex/gender and race/ethnicity, from those trials must be submitted not later than one year after the trial's primary completion date. Submission of results information can be delayed in certain circumstances for up to two additional years for trials

of products regulated by the FDA that are unapproved, unlicensed, or uncleared or for trials of products for which approval, licensure, or clearance of new use is being sought.

ARTICLE H.13. INCLUSION OF INDIVIDUALS ACROSS THE LIFESPAN AS PARTICIPANTS IN RESEARCH INVOLVING HUMAN SUBJECTS

Section 2038 of the 21st Century Cures Act, enacted December 13, 2016, enacts new provisions requiring NIH to address the consideration of age as an inclusion variable in research involving human subjects, to identify criteria for justification for any age-related exclusions in NIH research, and to provide data on the age of participants in clinical research studies. The NIH Policy and Guidelines on the Inclusion of Individuals Across the Lifespan as Participants in Research Involving Human Subjects applies to all NIH conducted or supported research involving human subjects, including research that is otherwise "exempt" in accordance with Sections 101(b) and 401(b) of 45 CFR 46 - Federal Policy for the Protection of Human Subjects.

Effective on all solicitations issued on or after January 25, 2019, individuals of all ages, including children (i.e. individuals under the age of 18) and older adults, must be included in all human subjects research, conducted or supported by the NIH, unless there are scientific or ethical reasons not to include them. The inclusion of individuals across the lifespan as subjects in research must be in compliance with all applicable subparts of 45 CFR 46 as well as with other pertinent federal laws and regulations.

The Contractor must address the age-appropriate inclusion or exclusion of individuals in the proposed research project. The Contractor must provide a description of plans for including individuals across the lifespan, including a rationale for selecting the specific age range justified in the context of the scientific question proposed. If individuals will be excluded from the research based on age, the contractor must provide acceptable justification for the exclusion.

The Contractor must submit cumulative data as prescribed in the Age Enrollment Report template on participant age at enrollment in monthly progress reports. Investigators planning to conduct research involving human subjects should design their studies in such a way that de-identified individual level participant data on sex/gender, race, ethnicity, and age at enrollment may be provided in progress reports.

ARTICLE H.14. POSTING CLINICAL TRIAL INFORMED CONSENT FORMS TO CLINICALTRIALS.GOV

The Revised Common Rule sections 46.102(b) and 46.116(h) requires Contractors to post one IRB-approved version of an Informed Consent Form that has been used to enroll participants on a public federal website designated for posting such Consent Forms. Contractors shall post the Informed Consent Form to the National Institutes of Health's (NIH's) clinical trials registry and results database ClinicalTrials.gov. Note: ClinicalTrials.gov only accepts Informed Consent Forms written in English; non-English language forms must be submitted to Regulations.gov. The Informed Consent Form must be posted after recruitment closes, and no later than 60 days after the final study visit. The Contracting Officer (CO) and/or Contracting Officer's Representative (COR) may permit or require redactions as appropriate.

ARTICLE H.15. REGISTRATION AND RESULTS REPORTING FOR APPLICABLE CLINICAL TRIALS CLINICALTRIALS.GOV

The Food and Drug Administration Amendments Act of 2007 (FDAAA), at: http://frwebgate.access.gpo.gov/cgi-bin/getdoc.cgi?dbname=110_cong_public_laws&docid=f:publ085.110.pdf , Title VIII, expands National Institutes of Health's (NIH's) clinical trials registry and results database known as ClinicalTrials.gov and imposes new requirements that apply to specified "applicable clinical trials," including those supported in whole or in part by NIH funds. FDAAA requires:

- the registration of certain "applicable clinical trials" (see Definitions http://grants.nih.gov/ClinicalTrials_fdaaa/definitions.htm) in ClinicalTrials.gov no later than 21 days after the first subject is enrolled; and

- the reporting of summary results information (including adverse events) no later than 1 year after the completion date (See Definitions at link above) for registered applicable clinical trials involving drugs that are approved under section 505 of the Food, Drug and Cosmetic Act (FDCA) or licensed under section 351 of the PHS Act, biologics, or of devices that are cleared under section 510k of FDCA.

The Contractor is the "Responsible Party" for the purposes of compliance with FDAAA which includes registration (and results reporting, if required) of the applicable clinical trial(s) performed under this contract in the Government database, ClinicalTrials.gov (http://www.ClinicalTrials.gov).

The contractor shall provide the "Responsible Party" with all essential data for timely compliance with ClinicalTrials.gov reporting requirements.

Additional information is available at: http://prsinfo.clinicaltrials.gov.

ARTICLE H.16. HUMAN MATERIALS

The acquisition and supply of all human specimen material (including fetal material) used under this contract shall be obtained by the Contractor in full compliance with applicable State and Local laws and the provisions of the Uniform Anatomical Gift Act in the United States, and no undue inducements, monetary or otherwise, will be offered to any person to influence their donation of human material.

ARTICLE H.17. HUMAN MATERIALS (ASSURANCE OF OHRP COMPLIANCE)

The acquisition and supply of all human specimen material (including fetal material) used under this contract shall be obtained by the Contractor in full compliance with applicable State and Local laws and the provisions of the Uniform Anatomical Gift Act in the United States, and no undue inducements, monetary or otherwise, will be offered to any person to influence their donation of human material.

The Contractor shall provide written documentation that all human materials obtained as a result of research involving human subjects conducted under this contract, by collaborating sites, or by subcontractors identified under this contract, were obtained with prior approval by the Office for Human Research Protections (OHRP) of an Assurance to comply with the requirements of 45 CFR 46 to protect human research subjects. This restriction applies to all collaborating sites without OHRP-approved Assurances, whether domestic or foreign, and compliance must be ensured by the Contractor.

Provision by the Contractor to the Contracting Officer of a properly completed "Protection of Human Subjects Assurance Identification/IRB Certification/Declaration of Exemption", Form OMB No. 0990-0263(formerly Optional Form 310), certifying IRB review and approval of the protocol from which the human materials were obtained constitutes the written documentation required. The human subject certification can be met by submission of a self designated form, provided that it contains the information required by the "Protection of Human Subjects Assurance Identification/IRB Certification/Declaration of Exemption", Form OMB No. 0990-0263(formerly Optional Form 310).

ARTICLE H.18. NIH POLICY ON ENHANCING REPRODUCIBILITY THROUGH RIGOR AND TRANSPARENCY

Contractors shall adhere to the NIH policy of enhancing reproducibility through rigor and transparency by addressing each of the four areas of the policy in performance of the Statement of Work and in publications, as applicable: 1) Scientific Premise; 2) Scientific Rigor; 3) Consideration of Relevant Biological Variables, including Sex; and 4) Authentication of Key Biological and/or Chemical Resources. This policy applies to all NIH funded research and development, from basic through advanced clinical studies. See NIH Guide Notice, NOT-OD-15-103, "Enhancing Reproducibility through Rigor and Transparency" and NOT-OD-15-102, "Consideration of Sex as a Biological Variable in NIH-funded Research" for more information. In addition, publications are expected to follow the guidance at http://www.nih.gov/research-training/rigor-reproducibility/principles-guidelines-reporting-preclinical-research, whether

preclinical or otherwise, as appropriate. More information is available at http://grants.nih.gov/reproducibility/index.htm, including FAQs and a General Policy Overview.

ARTICLE H.19. DATA SHARING IN LARGE-SCALE HUMAN OR NON-HUMAN GENOMIC DATA

The Contractor shall comply with the NIH "Genomic Data Sharing Policy" located at: https://osp.od.nih.gov/wp-content/uploads/NIH_GDS_Policy.pdf. The contractor shall submit and certify data obtained in the genomic data study to the data repository in accordance with the policy. The contractor shall also submit the data to the Contracting Officer and Contracting Officer's Representative.
Large-scale data include genome-wide association studies, single nucleotide polymorphisms arrays, and genome sequence, transcriptomic, metagenomic, epigenomic, and gene expression data, irrespective of funding level and funding mechanism.

ARTICLE H.20. SHARING HeLa CELL WHOLE GENOME SEQUENCE DATA AND FAMILY ACKNOWLEDGEMENT

All research using HeLa Cell Whole Genome Sequence data shall be conducted in accordance with NIH notice NOT-OD-13-099, entitled, "Notice of NIH Guidance on the Family Acknowledgement and Use of HeLa Cell Whole Genome Sequence Data" located at: https://grants.nih.gov/grants/guide/notice-files/NOT-OD-13-099.html. The Contractor shall submit HeLa Whole Genome Sequence Data generated under this contract to the database of Genotypes and Phenotypes (dbGaP) available at: http://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000640.v1.p1, in accordance with the HeLa Genome Data Use Agreement available at: https://dbgap.ncbi.nlm.nih.gov/aa/wga.cgi?view_pdf&stacc=phs000640.v1.p1.

The following acknowledgment, or a variation of it that has been reviewed by the HeLa Genome Data Access Working Group, shall be made in any dissemination of research findings:

"The genome sequence described/used in this research was derived from a HeLa cell line (URL to dbGaP). Henrietta Lacks, and the HeLa cell line that was established from her tumor cells without her knowledge or consent in 1951, have made significant contributions to scientific progress and advances in human health. We are grateful to Henrietta Lacks, now deceased, and to her surviving family members for their contributions to biomedical research. This study was reviewed by the NIH HeLa Genome Data Access Working Group."

Contact helagenome@nih.gov for acknowledgement variation requests.

ARTICLE H.21. NIH POLICY ON ENHANCING PUBLIC ACCESS TO ARCHIVED PUBLICATIONS RESULTING FROM NIH-FUNDED RESEARCH

NIH-funded investigators shall submit to the NIH National Library of Medicine's (NLM) PubMed Central (PMC) an electronic version of the author's final manuscript, upon acceptance for publication, resulting from research supported in whole or in part with direct costs from NIH. NIH defines the author's final manuscript as the final version accepted for journal publication, and includes all modifications from the publishing peer review process. The PMC archive will preserve permanently these manuscripts for use by the public, health care providers, educators, scientists, and NIH. The Policy directs electronic submissions to the NIH/NLM/PMC: http://www.pubmedcentral.nih.gov.

Additional information is available at http://grants.nih.gov/grants/guide/notice-files/NOT-OD-09-071.html and http://publicaccess.nih.gov.

ARTICLE H.22. NEEDLE EXCHANGE, HHSAR 352.270-12 (December 2015)

The Contractor shall not use any funds obligated under this contract to carry out any program of distributing sterile needles or syringes for the hypodermic injection of any illegal drug.

(End of clause)

ARTICLE H.23. ACKNOWLEDGEMENT OF FEDERAL FUNDING

The Contractor shall clearly state, when issuing statements, press releases, requests for proposals, bid solicitations and other documents describing projects or programs funded in whole or in part with Federal money: (1) the percentage of the total costs of the program or project which will be financed with Federal money; (2) the dollar amount of Federal funds for the project or program; and (3) the percentage and dollar amount of the total costs of the project or program that will be financed by nongovernmental sources.

ARTICLE H.24. CONTINUED BAN ON FUNDING ABORTION AND CONTINUED BAN ON FUNDING OF HUMAN EMBRYO RESEARCH, HHSAR 352.270-13 (December 2015)

a. The Contractor shall not use any funds obligated under this contract for any abortion.

b. The Contractor shall not use any funds obligated under this contract for the following:

    1. The creation of a human embryo or embryos for research purposes; or

    2. Research in which a human embryo or embryos are destroyed, discarded, or knowingly subjected to risk of injury of death greater than that allowed for research on fetuses in utero under 45 CFR part 46 and Section 498(b) of the Public Health Service Act (42 U.S.C. 289g(b)).

c. The term ``human embryo or embryos'' includes any organism, not protected as a human subject under 45 CFR part 46 as of the date of the enactment of this Act, that is derived by fertilization, parthenogenesis, cloning, or any other means from one or more human gametes of human diploid cells.

d. The Contractor shall not use any Federal funds for the cloning of human beings.

(End of clause)

ARTICLE H.25. LIMITATION ON USE OF FUNDS FOR PROMOTION OF LEGALIZATION OF CONTROLLED SUBSTANCES

The Contractor shall not use contract funds to support activities that promote the legalization of any drug or other substance included in schedule I of the schedules of controlled substances established under section 202 of the Controlled Substances Act, except for normal and recognized executive-congressional communications. This limitation shall not apply when the Government determines that there is significant medical evidence of a therapeutic advantage to the use of such drug or other substance or that federally sponsored clinical trials are being conducted to determine therapeutic advantage.

ARTICLE H.26. DISSEMINATION OF FALSE OR DELIBERATELY MISLEADING INFORMATION

The Contractor shall not use contract funds to disseminate information that is deliberately false or misleading.

ARTICLE H.27. CARE OF LIVE VERTEBRATE ANIMALS, HHSAR 352.270-5(b) (December 2015)

1. Before undertaking performance of any contract involving animal-related activities where the species is regulated by the United Sates Department of Agriculture (USDA), the Contractor shall register with the Secretary of Agriculture of the United States in accordance with 7 U.S.C. 2136 and 9 CFR 2.25 through 2.28. The Contractor shall furnish evidence of the registration to the Contracting Officer.
2. The Contractor shall acquire vertebrate animals used in research from a dealer licensed by the Secretary of Agriculture under 7 U.S.C. 2133 and 9 CFR 2.1 2.11, or from a source that is exempt from licensing under those sections.
3. The Contractor agrees that the care, use, and intended use of any live vertebrate animals in the performance of this contract shall conform with the Public Health Service (PHS) Policy on Humane Care and Use of Laboratory Animals (PHS Policy), the current Animal Welfare Assurance (Assurance), the Guide for the Care and Use of Laboratory Animals (National Academy Press, Washington, DC) and the pertinent laws and regulations of the United States Department of Agriculture (see 7 U.S.C. 2131 et seq. and 9 CFR subchapter A, Parts 1-4). In case of conflict between standards, the more stringent standard shall govern.
4. If at any time during performance of this contract, the Contracting Officer determines, in consultation with the Office of Laboratory Animal Welfare (OLAW), National Institutes of Health (NIH), that the Contractor is not in compliance with any of the requirements and standards stated in paragraphs (a) through (c)above, the Contracting Officer may immediately suspend, in whole or in part, work and further payments under this contract until the Contractor corrects the noncompliance. Notice of the suspension may be communicated by telephone and confirmed in writing. If the Contractor fails to complete corrective action within the period of time designated in the Contracting Officer's written notice of suspension, the Contracting Officer may, in consultation with OLAW, NIH, terminate this contract in whole or in part, and the Contractor's name may be removed from the list of those contractors with Animal Welfare Assurances.

Note: The Contractor may request registration of its facility and a current listing of licensed dealers from the Regional Office of the Animal and Plant Health Inspection Service (APHIS), USDA, for the region in which its research facility is located. The location of the appropriate APHIS Regional Office, as well as information concerning this program may be obtained by contacting the Animal Care Staff, USDA/APHIS, 4700 River Road, Riverdale, Maryland 20737 (Email: ace@aphis.usda.gov; Web site: (http://www.aphis.usda.gov/wps/portal/aphis/ourfocus/animalwelfare).

(End of clause)

ARTICLE H.28. ANIMAL WELFARE

All research involving live, vertebrate animals shall be conducted in accordance with the Public Health Service Policy on Humane Care and Use of Laboratory Animals (PHS Policy). The PHS Policy can be accessed at: http://grants1.nih.gov/grants/olaw/references/phspol.htm.

ARTICLE H.29. INTRODUCTION OF RODENTS AND RODENT PRODUCTS

No rodent or rodent product shall be delivered into the NIH, NIAID environment (NIH) directly, or through collaborative research or holding facilities under contract to NIAID except by permit. Direct shipments to NIH from a Division of Veterinary Resources (DVR), Office of Research Services (ORS) approved source will be considered exempt. Non-exempt sources must be approved by permit issued through the DVR, ORS. The permit must be obtained by the Contractor prior to the shipment to NIH of the rodents and/or rodent products. The Contractor must be sure that this permit exists and is current before transferring rodents or rodent products into the NIH, NIAID environment. Refusal or negligence to do so will be considered a material breach of contract and may be treated as any other such material breach. Applications for permits should be submitted by facsimile not less than 30 days prior (60 days in situations where quarantine is likely) to shipping date to: NIH Division of Veterinary Resources (DVR), Office of Research

Services (ORS), Building 14G, Service Rd. South, Room 102, BETHESDA MD 20892-5210, (301)496-2527, FAX: (301) 402-0352.

ARTICLE H.30. PROTECTION OF PERSONNEL WHO WORK WITH NONHUMAN PRIMATES

All Contractor personnel who work with nonhuman primates or enter rooms or areas containing nonhuman primates shall comply with the procedures set forth in NIH Policy Manual 3044-2, entitled, "Protection of NIH Personnel Who Work with Nonhuman Primates," located at the following URL:

http://oma.od1.nih.gov/manualchapters/intramural/3044-2/

ARTICLE H.31. RESTRICTION FROM USE OF LIVE VERTEBRATE ANIMALS

UNDER GOVERNING POLICY, FEDERAL FUNDS ADMINISTERED BY THE PUBLIC HEALTH SERVICE (PHS) SHALL NOT BE EXPENDED FOR RESEARCH INVOLVING LIVE VERTEBRATE ANIMALS WITHOUT PRIOR APPROVAL BY THE OFFICE OF LABORATORY ANIMAL WELFARE (OLAW), OF **AN ANIMAL WELFARE ASSURANCE THAT COMPLIES WITH THE PHS POLICY ON HUMANE CARE AND USE OF LABORATORY ANIMALS AND/OR A VALID INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) APPROVAL**. THIS RESTRICTION APPLIES TO ALL PERFORMANCE SITES (e.g. COLLABORATING INSTITUTIONS, SUBCONTRACTORS, SUBGRANTEES) WITHOUT OLAW-APPROVED ASSURANCES, WHETHER DOMESTIC OR FOREIGN.

ARTICLE H.32. OMB CLEARANCE

In accordance with HHSAR 352.211-3, Paperwork Reduction Act, the Contractor shall not proceed with surveys or interviews until such time as Office of Management and Budget (OMB) Clearance for conducting interviews has been obtained by the Contracting Officer's Representative (COR) and the Contracting Officer has issued written approval to proceed.

ARTICLE H.33. RESTRICTION ON PORNOGRAPHY ON COMPUTER NETWORKS

The Contractor shall not use contract funds to maintain or establish a computer network unless such network blocks the viewing, downloading, and exchanging of pornography.

ARTICLE H.34. GUN CONTROL

The Contractor shall not use contract funds in whole or in part, to advocate or promote gun control.

ARTICLE H.35. OPTION PROVISION

Unless the Government exercises its option pursuant to the Option Clause set forth in SECTION I., the contract will consist only of the Base Period of the Statement of Work as defined in Sections C and F of the contract. Pursuant to FAR Clause 52.217-7, Option for Increased Quantity-Separately Priced Line Item set forth in SECTION I. of this contract, the Government may, by unilateral contract modification, require the Contractor to perform additional options set forth in the Statement of Work and also defined in Sections C and F of the contract. If the Government exercises this option, notice must be given prior to the expiration date of this contract, and the estimated cost plus fixed fee of the contract will be increased as set forth in the ESTIMATED COST PLUS FIXED FEE Article in SECTION B of this contract.

ARTICLE H.36. SUBCONTRACTING PROVISIONS

a. **Small Business Subcontracting Plan**

1. The Small Business Subcontracting Plan, dated July 26, 2019 is attached hereto and made a part of this contract.
2. The failure of any Contractor or subcontractor to comply in good faith with FAR Clause 52.219-8, entitled "Utilization of Small Business Concerns" incorporated in this contract and the attached Subcontracting Plan, will be a material breach of such contract or subcontract and subject to the remedies reserved to the Government under FAR Clause 52.219-16 entitled, "Liquidated Damages-Subcontracting Plan."

b. **Subcontracting Reports**

The Contractor shall submit the following Subcontracting reports electronically via the "electronic Subcontracting Reporting System (eSRS) at http://www.esrs.gov.

1. Individual Subcontract Reports (ISR)

   Regardless of the effective date of this contract, the Report shall be due on the following dates for the entire life of this contract:

   April 30th
   October 30th
   Expiration Date of Contract

2. Summary Subcontract Report (SSR)

   Regardless of the effective date of this contract, the Summary Subcontract Report shall be submitted annually on the following date for the entire life of this contract:

   October 30th

For both the Individual and Summary Subcontract Reports, the Contract Specialist shall be included as a contact for notification purposes at the following e-mail address:

julie.rodriguez@nih.gov
Contract Specialist

ARTICLE H.37. ELECTRONIC AND INFORMATION TECHNOLOGY ACCESSIBILITY, HHSAR 352.239-74 (December 2015)

a. Pursuant to Section 508 of the Rehabilitation Act of 1973(29 U.S.C. 794d), as amended by the Workforce Investment Act of 1998, all electronic and information technology (EIT) supplies and services developed, acquired, or maintained under this contract or order must comply with the "Architectural and Transportation Barriers Compliance Board Electronic and Information Technology (EIT) Accessibility Standards" set forth by the Architectural and Transportation Barriers Compliance Board (also referred to as the "Access Board") in 36 CFR part 1194. Information about Section 508 is available at http://www.hhs.gov/web/508. The complete text of Section 508 Final Provisions can be accessed at http://www.access-board.gov/guidelines-and-standards/communications-and-it/about-the-section-508-standards.

b. The Section 508 accessibility standards applicable to this contract or order are identified in the Statement of Work or Specification or Performance Work Statement. The contractor must provide any necessary updates to the submitted HHS Product Assessment Template(s) at the end of each contract or order exceeding the simplified acquisition threshold (see FAR 2.101) when the contract or order duration is one year or less. If it is determined by the Government that EIT supplies and services provided by the Contractor do not conform to the described accessibility standards in the contract, remediation of the supplies or services to the level of conformance specified in the contract will be the responsibility of the Contractor at its own expense.

c. The Section 508 accessibility standards applicable to this contract are: (Contract staff must list applicable standards)

d. In the event of a modification(s) to this contract or order,which adds new EIT supplies or services or revises the type of, or specifications for, supplies or services, the Contracting Officer may require that the contractor submit

a completed HHS Section 508 Product Assessment Template and any other additional information necessary to assist the Government in determining that the EIT supplies or services conform to Section 508 accessibility standards. Instructions for documenting accessibility via the HHS Section 508 Product Assessment Template may be found under Section 508 policy on the HHS Web site: (http://www.hhs.gov/web/508). If it is determined by the Government that EIT supplies and services provided by the Contractor do not conform to the described accessibility standards in the contract, remediation of the supplies or services to the level of conformance specified in the contract will be the responsibility of the Contractor at its own expense.

e. If this is an Indefinite Delivery contract, a Blanket Purchase Agreement or a Basic Ordering Agreement, the task/delivery order requests that include EIT supplies or services will define the specifications and accessibility standards for the order. In those cases, the Contractor may be required to provide a completed HHS Section 508 Product Assessment Template and any other additional information necessary to assist the Government in determining that the EIT supplies or services conform to Section 508 accessibility standards. Instructions for documenting accessibility via the HHS Section 508 Product Assessment Template may be found at http://www.hhs.gov/web/508. If it is determined by the Government that EIT supplies and services provided by the Contractor do not conform to the described accessibility standards in the provided documentation, remediation of the supplies or services to the level of conformance specified in the contract will be the responsibility of the Contractor at its own expense.

(End of clause)

ARTICLE H.38. INSTITUTIONAL RESPONSIBILITY REGARDING INVESTIGATOR FINANCIAL CONFLICTS OF INTEREST

The Institution (includes any contractor, public or private, excluding a Federal agency) shall comply with the requirements of 45 CFR Part 94, Responsible Prospective Contractors, which promotes objectivity in research by establishing standards to ensure that Investigators (defined as the project director or principal Investigator and any other person, regardless of title or position, who is responsible for the design, conduct, or reporting of research funded under NIH contracts, or proposed for such funding, which may include, for example, collaborators or consultants) will not be biased by any Investigator financial conflicts of interest. 45 CFR Part 94 is available at the following Web site: : http://www.ecfr.gov/cgi-bin/text-idx?c=ecfr&SID=0af84ca649a74846f102aaf664da1623&rgn=div5&view=text&node=45:1.0.1.1.51&idno=45
As required by 45 CFR Part 94, the Institution shall, at a minimum:

a. Maintain an up-to-date, written, enforceable policy on financial conflicts of interest that complies with 45 CFR Part 94, inform each Investigator of the policy, the Investigator's reporting responsibilities regarding disclosure of significant financial interests, and the applicable regulation, and make such policy available via a publicly accessible Web site, or if none currently exist, available to any requestor within five business days of a request. A significant financial interest means a financial interest consisting of one or more of the following interests of the Investigator (and those of the Investigator's spouse and dependent children) that reasonably appears to be related to the Investigator's institutional responsibilities:

   1. With regard to any publicly traded entity, a significant financial interest exists if the value of any remuneration received from the entity in the twelve months preceding the disclosure and the value of any equity interest in the entity as of the date of disclosure, when aggregated, exceeds $5,000. Included are payments and equity interests;

   2. With regard to any non-publicly traded entity, a significant financial interest exists if the value of any remuneration received from the entity in the twelve months preceding the disclosure, when aggregated, exceeds $5,000, or when the Investigator (or the Investigator's spouse or dependent children) holds any equity interest; or

   3. Intellectual property rights and interests, upon receipt of income related to such rights and interest.

   Significant financial interests do not include the following:

1. Income from seminars, lectures, or teaching, and service on advisory or review panels for government agencies, Institutions of higher education, academic teaching hospitals, medical centers, or research institutes with an Institution of higher learning; and
2. Income from investment vehicles, such as mutual funds and retirement accounts, as long as the Investigator does not directly control the investment decisions made in these vehicles.

b. Require each Investigator to complete training regarding the Institution's financial conflicts of interest policy prior to engaging in research related to any NIH-funded contract and at least every four years. The Institution must take reasonable steps [see Part 94.4(c)] to ensure that investigators working as collaborators, consultants or subcontractors comply with the regulations.

c. Designate an official(s) to solicit and review disclosures of significant financial interests from each Investigator who is planning to participate in, or is participating in, the NIH-funded research.

d. Require that each Investigator who is planning to participate in the NIH-funded research disclose to the Institution's designated official(s) the Investigator's significant financial interest (and those of the Investigator's spouse and dependent children) no later than the date of submission of the Institution's proposal for NIH-funded research. Require that each Investigator who is participating in the NIH-funded research to submit an updated disclosure of significant financial interests at least annually, in accordance with the specific time period prescribed by the Institution during the period of the award as well as within thirty days of discovering or acquiring a new significant financial interest.

e. Provide guidelines consistent with the regulations for the designated official(s) to determine whether an Investigator's significant financial interest is related to NIH-funded research and, if so related, whether the significant financial interest is a financial conflict of interest. An Investigator's significant financial interest is related to NIH-funded research when the Institution, thorough its designated official(s), reasonably determines that the significant financial interest: Could be affected by the NIH-funded research; or is in an entity whose financial interest could be affected by the research. A financial conflict of interest exists when the Institution, through its designated official(s), reasonably determines that the significant financial interest could directly and significantly affect the design, conduct, or reporting of the NIH-funded research.

f. Take such actions as necessary to manage financial conflicts of interest, including any financial conflicts of a subcontractor Investigator. Management of an identified financial conflict of interest requires development and implementation of a management plan and, if necessary, a retrospective review and mitigation report pursuant to Part 94.5(a).

g. Provide initial and ongoing FCOI reports to the Contracting Officer pursuant to Part 94.5(b).

h. Maintain records relating to all Investigator disclosures of financial interests and the Institution's review of, and response to, such disclosures, and all actions under the Institution's policy or retrospective review, if applicable, for at least 3 years from the date of final payment or, where applicable, for the other time periods specified in 48 CFR Part 4, subpart 4.7, Contract Records Retention.

i. Establish adequate enforcement mechanisms and provide for employee sanctions or other administrative actions to ensure Investigator compliance as appropriate.

j. Complete the certification in Section K - Representations, Certifications, and Other Statements of Offerors titled "Certification of Institutional Policy on Financial Conflicts of Interest".

If the failure of an Institution to comply with an Institution's financial conflicts of interest policy or a financial conflict of interest management plan appears to have biased the design, conduct, or reporting of the NIH-funded research, the Institution must promptly notify the Contracting Officer of the corrective action taken or to be taken. The Contracting Officer will consider the situation and, as necessary, take appropriate action or refer the matter to the Institution for further action, which may include directions to the Institution on how to maintain appropriate objectivity in the NIH-funded research project.

The Contracting Officer and/or HHS may inquire at any time before, during, or after award into any Investigator disclosure of financial interests, and the Institution's review of, and response to, such disclosure, regardless of whether the disclosure resulted in the Institution's determination of a financial conflict of interests.. The Contracting Officer may require submission of the records or review them on site. On the basis of this review of records or other information that may be available, the Contracting Officer may decide that a particular financial conflict of interest will bias the objectivity of the NIH-funded research to such an extent that further corrective action is needed or that the Institution has not managed the financial conflict of interest in accordance with Part 94.6(b). The issuance of a Stop Work Order by the Contracting Officer may be necessary until the matter is resolved.

If the Contracting Officer determines that NIH-funded clinical research, whose purpose is to evaluate the safety or effectiveness of a drug, medical device, or treatment, has been designed, conducted, or reported by an Investigator with a financial conflict of interest that was not managed or reported by the Institution, the Institution shall require the Investigator involved to disclose the financial conflict of interest in each public presentation of the results of the research and to request an addendum to previously published presentations.

ARTICLE H.39. PUBLICATION AND PUBLICITY

In addition to the requirements set forth in HHSAR Clause **352.227-70, Publications and Publicity** incorporated by reference in SECTION I of this contract, the Contractor shall acknowledge the support of the National Institutes of Health whenever publicizing the work under this contract in any media by including an acknowledgment substantially as follows:

> "This project has been funded in whole or in part with Federal funds from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Department of Health and Human Services, under Contract No. 75N93019C00058"

a. **Advanced Copies of Press Releases**

Press releases shall be considered to include the public release of information to any medium, excluding peer-reviewed scientific publications. The contractor shall ensure that the Contracting Officer's Representative (COR) has received an advance copy of any press release related to this contract not less than four (4) working days prior to the issuance of the press release.

ARTICLE H.40. REPORTING MATTERS INVOLVING FRAUD, WASTE AND ABUSE

Anyone who becomes aware of the existence or apparent existence of fraud, waste and abuse in NIH funded programs is encouraged to report such matters to the HHS Inspector General's Office in writing or on the Inspector General's Hotline. The toll free number is **1-800-HHS-TIPS (1-800-447-8477).** All telephone calls will be handled confidentially. The website to file a complaint on-line is: http://oig.hhs.gov/fraud/hotline/ and the mailing address is:

US Department of Health and Human Services
Office of Inspector General
ATTN: OIG HOTLINE OPERATIONS
P.O. Box 23489
Washington, D.C. 20026

ARTICLE H.41. OBTAINING AND DISSEMINATING BIOMEDICAL RESEARCH RESOURCES

Unique research resources arising from NIH-funded research are to be shared with the scientific research community. NIH provides guidance, entitled, "Principles and Guidelines for Recipients of NIH Research Grants and Contracts on Obtaining and Disseminating Biomedical Research Resources: Final Notice," (Federal Register Notice, December 23, 1999 [64 FR 72090]), concerning the appropriate terms for disseminating and acquiring these research resources. This guidance, found at: http://www.gpo.gov/fdsys/pkg/FR-1999-12-23/pdf/99-33292.pdf is intended to help contractors ensure that the conditions they impose and accept on the transfer of research tools will facilitate further biomedical research, consistent with the requirements of the Bayh-Dole Act and NIH funding policy.

Note: For the purposes of this Article, the terms, "research tools", "research materials", and "research resources" are used interchangeably and have the same meaning.

a. Sharing of Model Organisms for Biomedical Research

The plan for sharing model organisms submitted by the Contractor is acceptable. The Contractor agrees to adhere to its plan and shall request prior approval of the Contracting Officer for any changes in its plan.

ARTICLE H.42. SHARING RESEARCH DATA

The data sharing plan submitted by the Contractor is acceptable. The Contractor agrees to adhere to its plan and shall request prior approval of the Contracting Officer for any changes in its plan.

The NIH endorses the sharing of final research data to serve health. This contract is expected to generate research data that must be shared with the public and other researchers. NIH's data sharing policy may be found at the following Web site:

http://grants.nih.gov/grants/guide/notice-files/NOT-OD-03-032.html

NIH recognizes that data sharing may be complicated or limited, in some cases, by institutional policies, local IRB rules, as well as local, state and Federal laws and regulations, including the Privacy Rule (see HHS-published documentation on the Privacy Rule at http://www.hhs.gov/ocr/). The rights and privacy of people who participate in NIH-funded research must be protected at all times; thus, data intended for broader use should be free of identifiers that would permit linkages to individual research participants and variables that could lead to deductive disclosure of the identity of individual subjects.

ARTICLE H.43. POSSESSION USE AND TRANSFER OF SELECT BIOLOGICAL AGENTS OR TOXINS

The work being conducted under this contract may involve the possession, use, or transfer of a select agent or toxin. The contractor shall not conduct work involving a Select Agent or Toxin under this contract until it and any associated subcontractor(s) comply with the following:

For prime or subcontract awards to ***domestic institutions*** that possess, use, and/or transfer a Select Agent or Toxin under this contract, the institution must comply with the provisions of 42 CFR part 73, 7 CFR part 331, and/or 9 CFR part 121 (http://www.selectagents.gov/Regulations.html) as required, before using NIH funds for work involving a *Select Agent or Toxin*. **No NIH funds can be used for research involving a *Select Agent or Toxin* at a domestic institution without a valid registration certificate.**

For prime or subcontract awards to ***foreign institutions*** that possess, use, and/or transfer a *Select Agent or Toxin*, before using NIH funds for any work directly involving a *Select Agent or Toxin*, the foreign institution must provide information satisfactory to the NIAID that safety, security, and training standards equivalent to those described in 42 CFR part 73, 7 CFR part 331, and/or 9 CFR part 121 are in place and will be administered on behalf of all *Select Agent or Toxin* work supported by these funds. The process for making this determination includes a site visit to the foreign laboratory facility by an NIAID representative. During this visit, the foreign institution must provide the following information: concise summaries of safety, security, and training plans;

names of individuals at the foreign institution who will have access to the Select Agent or Toxin and procedures for ensuring that only approved and appropriate individuals, in accordance with institution procedures, will have access to the Select Agents or Toxins under the contract; and copies of or links to any applicable laws, regulations, policies, and procedures applicable to that institution for the safe and secure possession, use, and/or transfer of select agents. Site visits to foreign laboratories are conducted every three years after the initial review. **No NIH funds can be used for work involving a *Select Agent or Toxin* at a foreign institution without written approval from the Contracting Officer.**

Prior to conducting a restricted experiment with a Select Agent or Toxin under this contract or any associated subcontract, the contractor must discuss the experiment with the Contracting Officer's Representative (COR) and request and obtain written approval from the Contracting Officer. **Domestic institutions** must submit to the Contracting Officer written approval from the CDC to perform the proposed restricted experiment. **Foreign institutions** require review by a NIAID representative. The prime contractor must contact the COR and the NIAID Office of International Extramural Activities (OIEA) at mailto:niaidforeignawards@niaid.nih.gov for guidance on the process used by NIAID to review proposed restricted experiments. The NIAID website provides an overview of the review process at http://funding.niaid.nih.gov/researchfunding/sci/biod/pages/saconproc.aspx . The Contracting Officer will notify the prime contractor when the process is complete. **No NIH funds can be used for a restricted experiment with a Select Agent or Toxin at either a domestic or foreign institution without written approval from the Contracting Officer.**
Listings of HHS and USDA select agents and toxins, and overlap select agents or toxins as well as information about the registration process for domestic institutions, are available on the Select Agent Program Web site at http://www.selectagents.gov/ and http://www.selectagents.gov/Select%20Agents%20and%20Toxins%20List.html.

For foreign institutions, see the NIAID Select Agent Award information:
(http://funding.niaid.nih.gov/researchfunding/sci/biod/pages/default.aspx).

ARTICLE H.44. PROHIBITION ON CONTRACTOR INVOLVEMENT WITH TERRORIST ACTIVITIES

The Contractor acknowledges that U.S. Executive Orders and Laws, including but not limited to E.O. 13224 and P.L. 107-56, prohibit transactions with, and the provision of resources and support to, individuals and organizations associated with terrorism. It is the legal responsibility of the Contractor to ensure compliance with these Executive Orders and Laws. This clause must be included in all subcontracts issued under this contract.

ARTICLE H.45. CONSTITUTION DAY

Each educational institution that receives Federal funds for a fiscal year shall hold an educational program on the United States Constitution on September 17 of such year for the students serviced by the educational institution in accordance with Public Law 108-447.

ARTICLE H.46. USE OF FUNDS FOR CONFERENCES, MEETINGS AND FOOD

The Contractor shall not use contract funds (direct or indirect) to conduct meetings or conferences in performance of this contract without prior written Contracting Officer approval.

In addition, the use of contract funds to purchase food for meals, light refreshments, or beverages is expressly prohibited.

PART II - CONTRACT CLAUSES

SECTION I - CONTRACT CLAUSES

ARTICLE I.1. GENERAL CLAUSES FOR A COST-REIMBURSEMENT CONTRACT WITH EDUCATIONAL INSTITUTIONS

This contract incorporates the following clauses by reference, with the same force and effect as if they were given in full text. Upon request, the Contracting Officer will make their full text available. Also, the full text of a clause may be accessed electronically as follows: FAR Clauses at: http://www.acquisition.gov/far/. HHSAR Clauses at: http://www.hhs.gov/policies/hhsar/subpart352.html.

a. FEDERAL ACQUISITION REGULATION (FAR) (48 CFR CHAPTER 1) CLAUSES:

FAR CLAUSE NO.	DATE	TITLE
52.202-1	Nov 2013	Definitions (Over the Simplified Acquisition Threshold)
52.203-3	Apr 1984	Gratuities (Over the Simplified Acquisition Threshold)
52.203-5	May 2014	Covenant Against Contingent Fees (Over the Simplified Acquisition Threshold)
52.203-6	Sep 2006	Restrictions on Subcontractor Sales to the Government (Over the Simplified Acquisition Threshold)
52.203-7	May 2014	Anti-Kickback Procedures (Over the Simplified Acquisition Threshold)
52.203-8	May 2014	Cancellation, Rescission, and Recovery of Funds for Illegal or Improper Activity (Over the Simplified Acquisition Threshold)
52.203-10	May 2014	Price or Fee Adjustment for Illegal or Improper Activity (Over the Simplified Acquisition Threshold)
52.203-12	Oct 2010	Limitation on Payments to Influence Certain Federal Transactions (Over $150,000)
52.203-17	Apr 2014	Contractor Employee Whistleblower Rights and Requirements to Inform Employees of Whistleblower Rights (Over the Simplified Acquisition Threshold)
52.203-99	Feb 2015	Prohibition on Contracting with Entities That Require Certain Internal Confidentiality Agreements (DEVIATION)
52.204-4	May 2011	Printed or Copied Double-Sided on Postconsumer Fiber Content Paper(Over the Simplified Acquisition Threshold)
52.204-10	Oct 2016	Reporting Executive Compensation and First-Tier Subcontract Awards ($30,000 or more)
52.204-13	Oct 2016	System for Award Management Maintenance
52.209-6	Oct 2015	Protecting the Government's Interest When Subcontracting With Contractors Debarred, Suspended, or Proposed for Debarment (Over $35,000)
52.215-2	Oct 2010	Audit and Records - Negotiation [Note: Applies to ALL contracts funded in whole or in part with Recovery Act funds, regardless of dollar value, AND contracts over the Simplified Acquisition Threshold funded exclusively with non-Recovery Act funds.], Alternate II (Aug 2016)
52.215-8	Oct 1997	Order of Precedence - Uniform Contract Format
52.215-10	Aug 2011	Price Reduction for Defective Certified Cost or Pricing Data (Over $750,000)
52.215-12	Oct 2010	Subcontractor Cost or Pricing Data (Over $750,000)
52.215-14	Oct 2010	Integrity of Unit Prices (Over the Simplified Acquisition Threshold)
52.215-15	Oct 2010	Pension Adjustments and Asset Reversions (Over $750,000)

FAR CLAUSE NO.	DATE	TITLE
52.215-18	Jul 2005	Reversion or Adjustment of Plans for Post-Retirement Benefits (PRB) other than Pensions
52.215-19	Oct 1997	Notification of Ownership Changes
52.215-21	Oct 2010	Requirements for Certified Cost or Pricing Data and Data Other Than Certified Cost or Pricing Data - Modifications
52.215-23	Oct 2009	Limitations on Pass-Through Charges (Over the Simplified Acquisition Threshold)
52.216-7	Jun 2013	Allowable Cost and Payment, Alternate II (Aug 2012)
52.216-11	Apr 1984	Cost Contract - No Fee
52.219-8	Nov 2016	Utilization of Small Business Concerns (Over the Simplified Acquisition Threshold)
52.219-9	Jan 2017	Small Business Subcontracting Plan (Over $700,000, $1.5 million for Construction)
52.219-16	Jan 1999	Liquidated Damages - Subcontracting Plan (Over $700,000, $1.5 million for Construction)
52.222-2	Jul 1990	Payment for Overtime Premium (Over the Simplified Acquisition Threshold) (Note: The dollar amount in paragraph (a) of this clause is $0 unless otherwise specified in the contract.)
52.222-3	Jun 2003	Convict Labor
52.222-21	Apr 2015	Prohibition of Segregated Facilities
52.222-26	Sep 2016	Equal Opportunity
52.222-35	Oct 2015	Equal Opportunity for Veterans ($150,000 or more)
52.222-36	Jul 2014	Equal Opportunity for Workers with Disabilities
52.222-37	Feb 2016	Employment Reports on Veterans ($150,000 or more)
52.222-40	Dec 2010	Notification of Employee Rights Under the National Labor Relations Act (Over the Simplified Acquisition Threshold)
52.222-50	Mar 2015	Combating Trafficking in Persons
52.222-54	Oct 2015	Employment Eligibility Verification (Over the Simplified Acquisition Threshold)
52.223-6	May 2001	Drug-Free Workplace
52.223-18	Aug 2011	Encouraging Contractor Policies to Ban Text Messaging While Driving
52.225-1	May 2014	Buy American - Supplies
52.225-13	Jun 2008	Restrictions on Certain Foreign Purchases
52.227-1	Dec 2007	Authorization and Consent, Alternate I (Apr 1984)
52.227-2	Dec 2007	Notice and Assistance Regarding Patent and Copyright Infringement
52.227-11	May 2014	Patent Rights - Ownership by the Contractor (Note: In accordance with FAR 27.303(b)(2), paragraph (e) is modified to include the requirements in FAR 27.303(b)(2)(i) through (iv). The frequency of reporting in (i) is annual.
52.227-14	Dec 2007	Rights in Data - General, Alternate IV (Dec 2007)
52.232-9	Apr 1984	Limitation on Withholding of Payments
52.232-20	Apr 1984	Limitation of Cost
52.232-23	May 2014	Assignment of Claims
52.232-25	Jul 2013	Prompt Payment, Alternate I (Feb 2002)

FAR CLAUSE NO.	***DATE***	***TITLE***
52.232-33	*Jul 2013*	*Payment by Electronic Funds Transfer--System for Award Management*
52.232-39	*Jun 2013*	*Unenforceability of Unauthorized Obligations*
52.233-1	*May 2014*	*Disputes*
52.233-3	*Aug 1996*	*Protest After Award, Alternate I (Jun 1985)*
52.233-4	*Oct 2004*	*Applicable Law for Breach of Contract Claim*
52.242-1	*Apr 1984*	*Notice of Intent to Disallow Costs*
52.242-4	*Jan 1997*	*Certification of Final Indirect Costs*
52.242-13	*Jul 1995*	*Bankruptcy (Over the Simplified Acquisition Threshold)*
52.244-2	*Oct 2010*	*Subcontracts (Over the Simplified Acquisition Threshold), Alternate I (June 2007)*
52.244-5	*Dec 1996*	*Competition in Subcontracting (Over the Simplified Acquisition Threshold)*
52.244-6	*Nov 2017*	*Subcontracts for Commercial Items*
52.245-1	*Apr 2012*	*Government Property, Alternate II (April 2012)*
52.245-9	*Apr 2012*	*Use and Charges*
52.246-23	*Feb 1997*	*Limitation of Liability (Over the Simplified Acquisition Threshold)*
52.249-6	*May 2004*	*Termination (Cost-Reimbursement)*
52.249-14	*Apr 1984*	*Excusable Delays*
52.253-1	*Jan 1991*	*Computer Generated Forms*

b. DEPARTMENT OF HEALTH AND HUMAN SERVICES ACQUISITION REGULATION (HHSAR) (48 CFR CHAPTER 3) CLAUSES:

HHSAR CLAUSE NO.	***DATE***	***TITLE***
352.203-70	*Dec 2015*	*Anti-Lobbying*
352.222-70	*Dec 2015*	*Contractor Cooperation in Equal Employment Opportunity Investigations*
352.227-70	*Dec 2015*	*Publications and Publicity*
352.233-71	*Dec 2015*	*Litigation and Claims*
352.237-75	*Dec 2015*	*Key Personnel*

[End of GENERAL CLAUSES FOR A NEGOTIATED COST-REIMBURSEMENT CONTRACT W_EDUCATIONAL INSTITUTION- Rev. 11/2017].

ARTICLE I.2. AUTHORIZED SUBSTITUTION OF CLAUSES

ARTICLE I.1. of this SECTION is hereby modified as follows:

a. **Alternate I** of FAR Clause **52.216-11, Cost Contract--No Fee** (April 1984), is added.

b. FAR Clauses **52.249-6, Termination (Cost-Reimbursement)** (May 2004) and **52.249-14, Excusable Delays** (April 1984), are deleted in their entirety and FAR Clause **52.249-5, Termination for Convenience of the Government (Educational and Other Nonprofit Institutions)** (August 2016), is substituted therefore.

ARTICLE I.3. Additional Contract Clauses

This contract incorporates the following clauses by reference, with the same force and effect, as if they were given in full text. Upon request, the Contracting Officer will make their full text available.

a. FEDERAL ACQUISITION REGULATION (FAR) (48 CFR CHAPTER 1) CLAUSES

1. FAR Clause **52.203-13, Contractor Code of Business Ethics and Conduct** (October 2015).

2. FAR Clause **52.203-14, Display of Hotline Poster(s)** (October 2015).

   ".....(3) Any required posters may be obtained as follows:

Poster(s)	Obtain From"
HHS Contractor Code of Ethics and Business Conduct Poster	http://oig.hhs.gov/fraud/report-fraud/OIG_Hotline_Poster.pdf

3. FAR Clause **52.204-14**, **Service Contract Reporting Requirements** (October 2016).

4. FAR Clause **52.209-10, Prohibition on Contracting With Inverted Domestic Corporations** (November 2015).

5. FAR Clause **52.210-1, Market Research** (April 2011).

6. FAR Clause **52.216-15, Predetermined Indirect Cost Rates** (April 1998).

7. FAR Clause **52.217-7, Option for Increased Quantity - Separately Priced Line Item** (March 1989).

   "....The Contracting Officer may exercise the option by written notice to the Contractor prior to contract completion date...."

8. FAR Clause **52.219-4, Notice of Price Evaluation Preference for HUBZone Small Business Concerns** (October 2014).

   "(c) Waiver of evaluation preference.....
   [ ] Offeror elects to waive the evaluation preference."

9. FAR Clause **52.219-28, Post-Award Small Business Program Rerepresentation** (July 2013).

10. FAR Clause **52.223-3, Hazardous Material Identification and Material Safety Data** (January 1997), with **Alternate I** (July 1995).

11. FAR Clause **52.227-14, Rights in Data - General** (May 2014).

12. **Alternate V** (December 2007), FAR Clause **52.227-14, Rights in Data--General** (May 2014).

Specific data items that are not subject to paragraph (j) include: none

13. FAR Clause **52.227-16, Additional Data Requirements** (June 1987).

14. FAR Clause **52.230-5, Cost Accounting Standards - Educational Institution** (August 2016).

15. FAR Clause **52.230-6, Administration of Cost Accounting Standards** (June 2010).

16. FAR Clause **52.242-3, Penalties for Unallowable Costs** (May 2014).

17. FAR Clause **52.243-2, Changes--Cost Reimbursement** (August 1987), **Alternate V** (April 1984).

18. FAR Clause **52.251-1, Government Supply Sources** (April 2012).

b. DEPARTMENT OF HEALTH AND HUMAN SERVICES ACQUISITION REGULATION (HHSAR) (48 CHAPTER 3) CLAUSES:

1. HHSAR Clause **352.208-70, Printing and Duplication** (December 2015)

2. HHSAR Clause **352.211-3, Paperwork Reduction Act** (December 2015)

3. HHSAR Clause **352.223-70, Safety and Health** (December 2015)

4. HHSAR Clause **352.231-70, Salary Rate Limitation** (December 2015)

Note: *The Salary Rate Limitation is at the Executive Level II Rate.*

See the following website for Executive Schedule rates of pay: https://www.opm.gov/policy-data-oversight/pay-leave/salaries-wages/.

(*For current year rates, click on Salaries and Wages/Executive Schedule/Rates of Pay for the Executive Schedule. For prior year rates, click on Salaries and Wages/select Another Year at the top of the page/Executive Schedule/Rates of Pay for the Executive Schedule. Rates are effective January 1 of each calendar year unless otherwise noted.*)

ARTICLE I.4. ADDITIONAL FAR CONTRACT CLAUSES INCLUDED IN FULL TEXT

This contract incorporates the following clauses in full text.

a. FEDERAL ACQUISITION REGULATION (FAR) (48 CFR CHAPTER 1) CLAUSES

1. FAR Clause 52.204-21, **Basic Safeguarding of Covered Contractor Information Systems** (June 2016)

a. *Definitions* . As used in this clause--

"Covered contractor information system" means an information system that is owned or operated by a contractor that processes, stores, or transmits Federal contract information.

"Federal contract information" means information, not intended for public release, that is provided by or generated for the Government under a contract to develop or deliver a product or service to the Government, but not including information provided by the Government to the public (such as on public Web sites) or simple transactional information, such as necessary to process payments.

"Information" means any communication or representation of knowledge such as facts, data, or opinions, in any medium or form, including textual, numerical, graphic, cartographic, narrative, or audiovisual (Committee on National Security Systems Instruction (CNSSI) 4009).

"Information system" means a discrete set of information resources organized for the collection, processing, maintenance, use, sharing, dissemination, or disposition of information (44 U.S.C. 3502).

"Safeguarding" means measures or controls that are prescribed to protect information systems.

b. Safeguarding requirements and procedures.

1. The Contractor shall apply the following basic safeguarding requirements and procedures to protect covered contractor information systems. Requirements and procedures for basic safeguarding of covered contractor information systems shall include, at a minimum, the following security controls:

i. Limit information system access to authorized users, processes acting on behalf of authorized users, or devices (including other information systems).

ii. Limit information system access to the types of transactions and functions that authorized users are permitted to execute.

iii. Verify and control/limit connections to and use of external information systems.

iv. Control information posted or processed on publicly accessible information systems.

v. Identify information system users, processes acting on behalf of users, or devices.

vi. Authenticate (or verify) the identities of those users, processes, or devices, as a prerequisite to allowing access to organizational information systems.

vii. Sanitize or destroy information system media containing Federal Contract Information before disposal or release for reuse.

viii. Limit physical access to organizational information systems, equipment, and the respective operating environments to authorized individuals.

ix. Escort visitors and monitor visitor activity; maintain audit logs of physical access; and control and manage physical access devices.

x. Monitor, control, and protect organizational communications (i.e., information transmitted or received by organizational information systems) at the external boundaries and key internal boundaries of the information systems.

xi. Implement subnetworks for publicly accessible system components that are physically or logically separated from internal networks.

xii. Identify, report, and correct information and information system flaws in a timely manner.

xiii. Provide protection from malicious code at appropriate locations within organizational information systems.

xiv. Update malicious code protection mechanisms when new releases are available.

xv. Perform periodic scans of the information system and real-time scans of files from external sources as files are downloaded, opened, or executed.

2. *Other requirements*. This clause does not relieve the Contractor of any other specific safeguarding requirements specified by Federal agencies and departments relating to covered contractor information systems generally or other Federal safeguarding requirements for controlled unclassified information (CUI) as established by Executive Order 13556.

c. *Subcontracts*. The Contractor shall include the substance of this clause, including this paragraph (c), in subcontracts under this contract (including subcontracts for the acquisition of commercial items, other than commercially available off-the-shelf items), in which the subcontractor may have Federal contract information residing in or transiting through its information system.

2. *FAR Clause* ***52.209-9, Updates of Publicly Available Information Regarding Responsibility Matters*** *(July 2013)*

As prescribed in 9.104-7(c), insert the following clause:

a. *The Contractor shall update the information in the Federal Awardee Performance and Integrity Information System (FAPIIS) on a semi-annual basis, throughout the life of the contract, by posting the required information in the System for Award Management (SAM) database at http://www.acquisition.gov.*

b. *As required by section 3010 of the Supplemental Appropriations Act, 2010 (Pub. L. 111-212), all information posted in FAPIIS on or after April 15, 2011, except past performance reviews, will be publicly available. FAPIIS consists of two segments--*

1. *The non-public segment, into which Government officials and the Contractor post information, which can only be viewed by--*

i. *Government personnel and authorized users performing business on behalf of the Government; or*

ii. *The Contractor, when viewing data on itself; and*

2. *The publicly-available segment, to which all data in the non-public segment of FAPIIS is automatically transferred after a waiting period of 14 calendar days, except for--*

i. *Past performance reviews required by subpart 42.15;*

ii. *Information that was entered prior to April 15, 2011; or*

iii. *Information that is withdrawn during the 14-calendar-day waiting period by the Government official who posted it in accordance with paragraph (c)(1) of this clause.*

c. *The Contractor will receive notification when the Government posts new information to the Contractor's record.*

1. *If the Contractor asserts in writing within 7 calendar days, to the Government official who posted the information, that some of the information posted to the non-public segment of FAPIIS is covered by a disclosure exemption under the Freedom of Information Act, the Government official who posted the information must within 7 calendar days remove the posting from FAPIIS and resolve the issue in accordance with agency Freedom of Information procedures, prior to reposting the releasable information. The contractor must cite 52.209-9 and request removal within 7 calendar days of the posting to FAPIIS.*
2. *The Contractor will also have an opportunity to post comments regarding information that has been posted by the Government. The comments will be retained as long as the associated information is retained, i.e., for a total period of 6 years. Contractor comments will remain a part of the record unless the Contractor revises them.*
3. *As required by section 3010 of Pub. L. 111-212, all information posted in FAPIIS on or after April 15, 2011, except past performance reviews, will be publicly available.*

d. *Public requests for system information posted prior to April 15, 2011, will be handled under Freedom of Information Act procedures, including, where appropriate, procedures promulgated under E.O. 12600.*

(End of clause)

PART III - LIST OF DOCUMENTS, EXHIBITS AND OTHER ATTACHMENTS

SECTION J - LIST OF ATTACHMENTS

The following documents are attached and incorporated in this contract:

1. Statement of Work

Statement of Work, dated August 1, 2019, 2 pages.

2. Invoice/Financing Request and Contract Financial Reporting Instructions for NIH Cost-Reimbursement Type Contracts, NIH(RC)-4

Invoice/Financing Request and Contract Financial Reporting Instructions for NIH Cost-Reimbursement Type Contracts, NIH(RC)-4, (7/13), 6 pages.

3. Cumulative Inclusion Enrollment Report

PHS Human Subjects and Clinical Trials Information Form/Cumulative Inclusion Enrollment Report, Located at:

https://oamp.od.nih.gov/sites/default/files/DGS/contracting-forms/humansubjectstudy-v1.0.pdf

4. Small Business Subcontracting Plan

Small Business Subcontracting Plan, dated July 26, 2019, 7 pages.

5. Safety and Health

Safety and Health, HHSAR Clause 352.223-70, (12/15), 2 pages.

6. Disclosure of Lobbying Activities, SF-LLL

Disclosure of Lobbying Activities, SF-LLL, dated 7/97, 2 pages.

PART IV - REPRESENTATIONS AND INSTRUCTIONS

SECTION K - REPRESENTATIONS AND CERTIFICATIONS

The following documents are incorporated by reference in this contract:

1. FAR Clause 52.204-19 **Incorporation by Reference of Representations and Certifications** (December 2014).

   The Contractor's representations and certifications, including those completed electronically via the System for Award Management (SAM), are incorporated by reference into the contract.

   (End of clause)

END of the SCHEDULE

(CONTRACT)

Statement of Work

Independently and not as an agent of the Government, the Contractor shall furnish all the necessary services, qualified personnel, material, equipment, and facilities, not otherwise provided by the Government as needed to perform the SOW below:

A. Scope of Work

The Contractor shall develop EIDD-2801, the 5'-isopropylester prodrug of the broadly active antiviral ribonucleoside analogue EIDD-1931, for the treatment of influenza infections.

B. Technical Requirements

The Contractor shall perform work as set forth below, and the related tasks as approved in the Product Development Plan and Work Plan identified in Article C.2.

1. Base (CLIN 1) – Prepare and Submit IND for Phase 1 Safety Trial

a. Prepare SAD/MAD protocol and investigational brochure
b. Manufacture placebo clinical supplies for SAD/MAD and flu challenge studies
c. Develop and validate bioanalytical methods for human plasma and urine to support SAD/MAD study
d. Prepare and submit IND
e. Initiate clinical site preparation, such as site assessment, kick-off meeting, and protocol development
f. Submit deliverables in accordance with the Reporting Requirements and Delivery Schedule

2. Option One (CLIN 2) – Conduct Phase 1 SAD/MAD Safety Trial

a. Complete clinical start-up activities, such as data management, site audit, and ship clinical supplies
b. Enroll and conduct Phase 1 FIH SAD/MAD study through last patient – last visit
c. Produce draft tables, figures, and listings (TFLs) of clinical data for initiation of the Phase 2a POC influenza challenge study
d. Write Clinical Study Report (CSR) and PK report and submit necessary documents to regulatory agencies
e. Submit deliverables in accordance with Reporting Requirements and Delivery Schedule

3. Option Two (CLIN 3) – Prepare Phase 2a Flu Challenge

a. Manufacture EIDD-2810 drug product for Phase 2a POC study and maintain stability studies
b. Write the Phase 2a POC clinical study protocol, informed consent form and other study specific documents working interactively with DMID
c. Prepare and submit necessary documents to European regulatory agencies including notification of FDA about Phase 2 trial in Europe
d. Submit deliverables in accordance with Reporting Requirements and Delivery Schedule

4. Option Three (CLIN 4) – Conduct Phase 2a Flu Challenge Trial

a. Continue ongoing stability studies for drug substance and drug product
b. Enroll, conduct, and complete Phase 2a POC influenza challenge study
c. Produce draft tables, figures, and listings (TFLs) of clinical data
d. Write Clinical Study Report (CSR) and PK report and submit necessary documents to regulatory agencies
e. Submit deliverables in accordance with Reporting Requirements and Delivery Schedule

INVOICE/FINANCING REQUEST AND CONTRACT FINANCIAL REPORTING INSTRUCTIONS FOR NIH COST-REIMBURSEMENT CONTRACTS, NIH(RC)-4

Format: Submit payment requests on the Contractor's self-generated form in the manner and format prescribed herein and as illustrated in the Sample Invoice/Financing Request. Standard Form 1034, Public Voucher for Purchases and Services Other Than Personal, may be used in lieu of the Contractor's self-generated form provided it contains all of the information shown on the Sample Invoice/Financing Request. DO NOT include a cover letter with the payment request.

Number of Copies: Submit payment requests in the quantity specified in the Invoice Submission Instructions in Section G of the Contract Schedule.

Frequency: Payment requests shall not be submitted more frequently than once every two weeks in accordance with the Allowable Cost and Payment Clause incorporated into this contract. Small business concerns may submit invoices/financing requests more frequently than every two weeks.

Cost Incurrence Period: Costs incurred must be within the contract performance period or covered by precontract cost provisions.

Billing of Costs Incurred: : If billed costs include (1) costs of a prior billing period, but not previously billed, or (2) costs incurred during the contract period and claimed after the contract period has expired, the Contractor shall cite the amount(s) and month(s) in which the costs were incurred.

Contractor's Fiscal Year: Prepare payment requests in such a manner that the Government can identify costs claimed with the Contractor's fiscal year.

Currency: All NIH contracts are expressed in United States dollars. When the Government pays in a currency other than United States dollars, billings shall be expressed, and payment by the Government shall be made, in that other currency at amounts coincident with actual costs incurred. Currency fluctuations may not be a basis of gain or loss to the Contractor. Notwithstanding the above, the total of all invoices paid under this contract shall not exceed the United States dollars authorized.

Costs Requiring Advance Approval: Costs requiring advance approval by the Contracting Officer, which are not set forth in the Contract Schedule shall be identified by the Contracting Officer's Authorization (COA) Number as a separate expenditure category on the payment request. In addition, the Contractor shall show any cost limitation or ceiling set forth in the Contract Schedule, i.e. an Advance Understanding, as a separate expenditure category on the payment request.

Invoice/Financing Request Identification: Identify each payment as either:

(a) **Interim Invoice/Contract Financing Request:** These are interim payment requests submitted during the contract performance period.

(b) **Completion Invoice:** Submit the completion invoice promptly upon completion of the work, but no later than one year from the contract completion date, or within 120 days after settlement of the final indirect cost rates covering the year in which the contract is physically complete (whichever date is later). The Contractor shall submit the completion invoice when all costs have been assigned to the contract and all performance provisions have been completed.

(c) **Final Invoice:** A final invoice may be required after the amounts owed have been settled between the Government and the Contractor (e.g., resolution of all suspensions and audit exceptions).

Preparation and Itemization of the Invoice/Financing Request:

The Contractor shall furnish the information set forth in the instructions below. The instructions are keyed to the entries on the Sample Invoice/Financing Request. ***All information must be legible or the invoice will be considered improper and returned to the Contractor.***

(a) **Designated Billing Office Name and Address:** Enter the designated billing office name and address, as identified in the Invoice Submission Instructions in Section G of the Contract Schedule.

(b) **Contractor's Name, Address, Point of Contact, TIN, and DUNS or DUNS+4 Number:** Show the Contractor's name and address exactly as they appear in the contract. Any invoice identified as improper will be sent to this address. Also include the name, title, phone number, and e-mail address of the Point of Contact in case of questions. If the remittance name differs from the legal business name, both names must appear on the invoice. Provide the Contractor's Federal Taxpayer Identification Number (TIN) and Data Universal Numbering System (DUNS) or DUNS+4 number. The DUNS number must identify the Contractor's name and address exactly as stated in the contract, and as registered in the System for Award Management (SAM) database.

When an approved assignment of claims has been executed, the Contractor shall provide the same information for the assignee as is required for the Contractor (i.e., name, address, point of contact, TIN, and DUNS number), with the remittance information clearly identified as such.

(c) **Invoice/Financing Request Number:** Identify each payment request by a unique invoice number, which can only be used one time regardless of the number of contracts or orders held by an organization. For example, if a contractor has already submitted invoice number 05 on one of its contracts or orders, it cannot use that same invoice number on any other contract or order. Payment requests with duplicate invoice numbers will be considered improper and returned to the contractor.

The NIH does not prescribe a particular numbering format but suggests using a job or account number for each contract and order followed by a sequential invoice number (example: 8675309-05). Invoice numbers are limited to 30 characters. There are no restrictions on the use of special characters, such as colons, dashes, forward slashes, or parentheses.

If all or part of an invoice is suspended and the contractor chooses to reclaim those costs on a supplemental invoice, the contractor may use the same unique invoice number followed by an alpha character, such as "R" for revised (example: 8675309-05R).

(d) **Date Invoice/Financing Request Prepared:** Insert the date the payment request is prepared.

(e) **Contract Number and Order Number (if applicable):** Insert the contract number and order number (as applicable).

(f) **Contract Title:** Insert the contract title exactly as it appears on the cover page of the contract and/or Section G of the Contract Schedule.

(g) **Current Contract Period of Performance:** Insert the contract start date/effective date through the current completion date of the contract.

(h) **Total Estimated Cost of Contract/Order:** Insert the total estimated cost of the contract, exclusive of fee. If billing under an order, insert the total estimated cost of the order, exclusive of fee. For contracts/orders with options or incremental funding provisions, enter the amount currently obligated and available for payment.

(i) **Total Fixed-Fee:** Insert the total fixed-fee (where applicable). For contracts/orders with options or incremental funding provisions, enter the amount currently obligated and available for payment (where applicable). **Note:** *If the contract provides for another type of Fee, i.e. Award or Incentive Fee, insert the amount available to be earned as identified in the contract and indicate the type of fee to be billed on the payment request.*

(j) **Two-Way/Three-Way Match:** Identify whether payment is to be made using a two-way or three-way match. To determine required payment method, refer to the Invoice Submission Instructions in Section G of the Contract Schedule.

(k) **Office of Acquisitions:** Insert the name of the Office of Acquisitions, as identified in the Invoice Submission Instructions in Section G of the Contract Schedule.

(l) **Central Point of Distribution:** Insert the Central Point of Distribution, as identified in the Invoice Submission Instructions in Section G of the Contract Schedule.

(m) **Billing Period:** Insert the beginning and ending dates (month, day, and year) of the period in which costs were incurred and for which reimbursement is claimed.

(n) **Amount Billed - Current Period:** Insert the amount claimed for the current billing period by major cost element, including any adjustments and fee. If the Contract Schedule contains separately priced line items, identify the contract line item(s) on the payment request and include a separate breakdown (by major cost element) for each line item.

(o) **Amount Billed - Cumulative:** Insert the cumulative amounts claimed by major cost element, including any adjustments and fee. If the Contract Schedule contains separately priced line items, identify the contract line item(s) on the payment request and include a separate breakdown (by major cost element) for each line item.

(p) **Direct Costs:** Insert the major cost elements. For each element, consider the application of the paragraph entitled "Costs Requiring Prior Approval" on page 1 of these instructions.

1) **Direct Labor:** Include salaries and wages paid (or accrued) for direct performance of the contract.

For Level of Effort contracts only, the Contractor shall provide the following information on a separate sheet of paper attached to the payment request:

- hours or percentage of effort and cost by labor category (as specified in the Level of Effort Article in Section F of the Contract Schedule) for the current billing period, and

- hours or percentage of effort and cost by labor category from contract inception through the current billing period. (NOTE: The Contracting Officer may require the Contractor to provide additional breakdown for direct labor, such as position title, employee name, and salary or hourly rate.)

2) **Fringe Benefits:** List any fringe benefits applicable to direct labor and billed as a direct cost. Cite the rate(s) used to calculate fringe benefit costs, if applicable.

3) **Accountable Personal Property:** Include permanent research equipment and general purpose equipment having a unit acquisition cost of $1,000 or more, with a life expectancy of more than two years, and sensitive property regardless of cost (see the HHS *Contractor's Guide for Contract of Government Property*). Show permanent research equipment separate from general purpose equipment.

   On a separate sheet of paper attached to the payment request, list each item for which reimbursement is requested. Precede the item with an asterisk (*) if the equipment is below the $1,000 approval level. Include reference to the following (as applicable):

   - item number for the specific piece of equipment listed in the Property Schedule, and,
   - Contracting Officer Authorization (COA) number, if the equipment is not covered by the Property Schedule.

   The Contracting Officer may require the Contractor to provide further itemization of property having specific limitations set forth in the contract.

4) **Materials and Supplies:** Include equipment with unit costs of less than $1,000 or an expected service life of two years or less, and consumable material and supplies regardless of amount.

5) **Premium Pay:** List remuneration in excess of the basic hourly rate.

6) **Consultant Fee:** List fees paid to consultants. Identify consultant by name or category as set forth in the contract or COA, as well as the effort (i.e., number of hours, days, etc.) and rate billed.

7) **Travel:** Include domestic and foreign travel. Foreign travel is travel outside of the United States and its territories and possessions. However, for an organization located outside the United States and its territories and possessions, foreign travel means travel outside that country. Foreign travel must be billed separately from domestic travel.

8) **Subcontract Costs:** List subcontractor(s) by name and amount billed.

9) **Other:** List all other direct costs in total unless exceeding $1,000 in amount. If over $1,000, list cost elements and dollar amounts separately. If the contract contains restrictions on any cost element, that cost element must be listed separately.

(q) **Cost of Money (COM):** Cite the COM factor and base in effect during the time the cost was incurred and for which reimbursement is claimed.

(r) **Indirect Costs:** Identify the indirect cost base (IDC), indirect cost rate, and amount billed for each indirect cost category.

(s) **Fixed-Fee:** Cite the formula or method of computation for fixed-fee, if applicable. The fixed-fee must be claimed as provided for by the contract. ***Note:*** *If the contract provides for another type of Fee, i.e. Award or Incentive Fee, provide the same documentation for the amount claimed.*

(t) **Total Amounts Claimed:** Insert the total amounts claimed for the current and cumulative periods.

(u) **Adjustments:** Include amounts conceded by the Contractor, outstanding suspensions, and/or disapprovals subject to appeal.

(v) **Grand Totals**

(w) **Certification:** The Contractor shall include the following certification at the bottom of each payment request:

"Pursuant to authority vested in me, I certify that this voucher is correct and proper for payment."

Note: *The contract may require additional certifications (See Invoice Submission Instructions in Section G of the Contract Schedule)*

The Contracting Officer may require the Contractor to submit detailed support for costs claimed on one or more interim payment requests.

FINANCIAL REPORTING INSTRUCTIONS:

These instructions correspond to the Columns on the Sample Invoice/Financing Request.

Column A - Expenditure Category: Enter the expenditure categories required by the contract.

Column B - Cumulative Percentage of Effort/Hrs. - Negotiated: Enter the percentage of effort or number of hours agreed to for each employee or labor category listed in Column A.

Column C - Cumulative Percentage of Effort/Hrs. - Actual: Enter the percentage of effort or number of hours worked by each employee or labor category listed in Column A.

Column D - Amount Billed - Current: Enter amounts billed during the current period.

Column E - Amount Billed - Cumulative: Enter the cumulative amounts to date.

Column F - Cost at Completion: Enter data only when the Contractor estimates that a particular expenditure category will vary from the amount negotiated. Realistic estimates are essential.

Column G - Contract Amount: Enter the costs agreed to for all expenditure categories listed in Column A.

Column H - Variance (Over or Under): Show the difference between the estimated costs at completion (Column F) and negotiated costs (Column G) when entries have been made in Column F. This column need not be filled in when Column F is blank. When a line item varies by plus or minus 10 percent, i.e., the percentage arrived at by dividing Column F by Column G, an explanation of the variance should be submitted. In the case of an overrun (net negative variance), this submission **shall not** be deemed as notice under the Limitation of Cost Clause in the contract.

Modifications: List all new modification(s) (not previously reported) in the amount negotiated for an item in the appropriate cost category.

Expenditures Not Negotiated: An expenditure for an item for which no amount was negotiated (e.g., at the discretion of the Contractor in performance of its contract) should be listed in the appropriate cost category and all columns filled in, except for G. Column H will of course show a 100 percent variance and will be explained along with those identified under H above.

SAMPLE INVOICE/FINANCING REQUEST AND CONTRACT FINANCIAL REPORT

(a) Designated Billing Office Name and Address:
National Institutes of Health
Office of Financial Management
Commercial Accounts
2115 East Jefferson Street, Room 4B432, MSC 8500
Bethesda, MD 20892-8500

(b) Contractor's Name, Address, Point of Contact, TIN, and DUNS or DUNS+4 Number:
ABC CORPORATION
100 Main Street
Anywhere, U.S.A. Zip+4
Name, Title, Phone Number, and E-mail Address of Contractor's Point of Contact.
DUNS or DUNS+4: _____
TIN: _____

(c) Invoice/Financing Request No.: _____
(d) Date Invoice/Financing Request Prepared: _____
(e) Contract No. and Order No. (if applicable): _____
(f) Contract Title: _____
(g) Current Contract Period of Performance: _____
(h) Total Estimated Cost of Contract/Order: _____
(i) Total Fixed Fee (if applicable):_____

(j) Two-Way Match: ____
Three-Way Match: ____
(k) Office of Acquisitions:_____
(l) Central Point of Distribution:_____

(m) This invoice/financing request represents reimbursable costs for the period from _____ to _____.

	Cumulative % of Effort/Hrs		Amount Billed				
Expenditure Category* A	Neg. B	Actual C	(n) Current D	(o) Cum E	Cost at Comp F	Contract Value G	Variance H
(p) Direct Costs:							
(1) Direct Labor							
(2) Fringe Benefits __%							
(3) Accountable Property							
(4) Materials & Supplies							
(5) Premium Pay							
(6) Consultant Fees							
(7) Travel							
(8) Subcontracts							
(9) Other							
Total Direct Costs							
(q) Cost of Money ____%							
(r) Indirect Costs ____%							
(s) Fixed Fee ____%							
(t) Total Amount Claimed							
(u) Adjustments							
(v) Grand Totals							

"Pursuant to authority vested in me, I certify that this voucher is correct and proper for payment."

_____ _____
(Name of Official) (Title)

*Attach details as specified in the contract or requested by the Contracting Officer

Cumulative Inclusion Enrollment Report

This report format should NOT be used for collecting data from study participants.

Study Title:

Comments:

Racial Categories	Ethnic Categories									Total
	Not Hispanic or Latino			Hispanic or Latino			Unknown/Not Reported Ethnicity			
	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	
American Indian/ Alaska Native										0
Asian										0
Native Hawaiian or Other Pacific Islander										0
Black or African American										0
White										0
More Than One Race										0
Unknown or Not Reported										0
Total	0	0	0	0	0	0	0	0	0	0

Solicitation/Contract No.: HHS-NIH-NIAID-BAA 2018	MOD No. (If applicable): Not Applicable

Title of Acquisition:
The Development of EIDD-2801, the 5'-Isopropylester Pro-Drug of the Broadly Active Antiviral Ribonucleoside Analog EIDD-9131, for the Treatment of Influenza Infections.

Contractor's Name: Emory University

Period of Performance: 9/1/2019 – 12/31/2022	Total Contract Amount (including options, and any modifications if this submission is due to a modification): $ (b) (4)
Total Modification Amount: (if applicable) $ Not Applicable	Base Period (if there are options): $ 1,637,319.00
Option 1 (if applicable): $ (b) (4)	Option 2 (if applicable): $ (b) (4)
Option 3 (if applicable): $	Option 4 (if applicable): $0.00
Option 5 (if applicable): $ 0.00	Option 6 (if applicable): $0.00
Contracting Officer/Specialist Name: George W. Kennedy, Contracting Officer	Tel & Fax: 240-689-5170
OPDIV/Division/Branch (including location): (NIAID)	Email: kennedyg@mail.nih.gov

SUBCONTRACT PLAN REQUIREMENTS- *Failure to include the essential information of FAR Subpart 19.7 may be cause for either a delay in acceptance or the rejection of a bid or offer when a subcontracting plan is required. "SUBCONTRACT," as used in this clause, means any agreement (other than one involving an employer-employee relationship) entered into by a Federal Government prime contractor or subcontractor requesting supplies or services required for performance of the contract or subcontract.*

If assistance is needed to locate small business sources, contact the Small Business Specialist (SBS) supporting the OPDIV. SBS contact information is located on the OSDBU website (http://www.hhs.gov/about/smallbusiness/osdbustaff.html) or you may contact the OSDBU headquarters at (202) 690-7300.

HHS currently has the following subcontracting goals for Fiscal Year **2017**:

Type of Concern	Goal (%)
Small Business	33.00%
Small Disadvantaged Business, including 8(a) Program Participants, Alaska Native Corporations (ANC) and Indian Tribes	5.00%
Women Owned Small Business	5.00%
Service Disabled Veteran Owned Small Business	3.00%
HUBZone	3.00%

For this procurement, or modification, HHS expects all proposed subcontracting plans to contain at a minimum the aforementioned percentages.

1. Type of Plan (check one):

Individual x ***Master** (Addendum) ☐ ***Commercial** ☐

*When Master is checked, Individual must also be checked and submission must include both.
*If Commercial is checked, please stop here and attach a copy of your commercial plan.

For Informational Purposes:

Individual plan - all elements developed specifically for this contract and applicable for the full term of this contract.

Master plan - goals developed for this contract, all other elements standardized and approved by a lead agency Federal Official; must be renewed every three years and contractor must provide copy of lead agency approval.

Commercial products/service plan - goals are negotiated with the initial agency on a company-wide basis rather than for individual contracts; this plan applies to the entire production of commercial service or items or a portion thereof. The contractor sells

	commercial products and services customarily used for non-government purposes. The plan is effective during the offeror's fiscal year.

2. Subcontracting Goal Data: (FAR19.702(a)(1-3) & FAR 52.219-9(d)(1)) Below indicate the dollar and percentage goals for Small Business (SB), Small Disadvantaged (SDB) including Alaska Native Corporations and Indian Tribes, Women-owned and Economically Disadvantaged Women-Owned (WOSB), Historically Underutilized Business Zone (HUBZone), Veteran Owned Small Business (VOSB), Service-Disabled Veteran-Owned (SDVOSB) Small Businesses and "Other than Small Business" (Other) as subcontractors. Indicate the base year and each option year, as specified in FAR 19.704. If any contract has more than four options, please attach additional sheets which illustrate dollar amounts and percentages. **PLEASE NOTE: Zero dollars is not an acceptable goal for the SB, SDB, WOSB, HUBZone, VOSB or SDVOSB categories since this does not demonstrate a good faith effort throughout the period of performance of the contract.**	**% of Total Contract $** If required by CO
For Individual Plans complete a(1): **(FAR 52.219-9(d)(2)(i))** a(1). Total dollars planned to be subcontracted under this contract is: (b) (4) If your contract includes options, please include the break down here: Base Period: (b) (4) Option Period 1: $ (b) (4) Option Period 2: $ Option Period 3: $ Option Period 4: $ Option Period 5: $ Option Period 6: $	For Individual Plan 100 % If options are applicable: Base: 100 % OPT 1: 100 % OPT 2: 100 % OPT 3: 100 % OPT 4: 100 %
b. Total dollars planned to be subcontracted to small business concerns (including ANC and Indian tribes)- [Percentage of 2a.]: **(FAR 52.219-9(d)(2)(ii))** (b) (4) and 33 % If your contract includes options, please include the break down here: Base: $ (b) (4) OPT 1: (b) (4) and 33% OPT 2 and 33 % OPT 3: and 33% OPT 4: $ and 33% OPT 5 and 33% OPT 6 $	33% If options are applicable: Base: 33% OPT 1: 33% OPT 2: 33% OPT 3: 33% OPT 4: 33% OPT 5: 33% OPT 6: 33%
c. Total dollars planned to be subcontracted to veteran-owned small business concerns- [Percentage of 2.a.]: **(FAR 52.219-9(d)(2)(iii))** $ (b) (4) and 3% If your contract includes options, please include the break down here: Base: (b) (4) OPT 1: (b) (4) and 3% OPT 2: 3% OPT 3: and 3 % and 3 % OPT 4 $ and 3 % OPT 5 $ OPT 6 $	3% If options are applicable: Base: 3% OPT 1: 3% OPT 2: 3% OPT 3: 3% OPT 4: 3% OPT 5: 3% OPT 6: 3%

and

d. Total dollars planned to be subcontracted to service-disabled veteran-owned small business - [Percentage of 2.a.]: **(FAR 52.219-9(d)(2)(iv))**

(b) (4) and

If your contract includes options, please include the break down here: 3%

Base: (b) (4)

OPT 1: (b) (4) and

OPT 2: and 3 %

OPT 3: and 3 %

OPT 4 $ and 3 %

OPT 5 $ and 3 %

OPT 6 $ and

3%

If options are applicable:
Base: 3%
OPT 1: 3%
OPT 2: 3%
OPT 3: 3%
OPT 4: 3%
OPT 5: 3%
OPT 6: 3%

e. Total dollars planned to be subcontracted to HUBZone small business concerns - [Percentage of 2.a.]: **(FAR 52.219-9(d)(2)(v))**

and

(b) (4) 3 %

If your contract includes options, please include the break down here:

Base: (b) (4) and

OPT 1: (b) (4) 3 %

OPT 2: and 3 %

OPT 3: 3 %

OPT 4 $ and 3 %

OPT 5 $ 3 %

OPT 6 $ and

3%

If options are applicable:
Base: 3%
OPT 1: 3%
OPT 2: 3%
OPT 3: 3%
OPT 4: 3%
OPT 5: 3%
OPT 6: 3%

f. Total dollars planned to be subcontracted to small disadvantaged business concerns (including ANCs and Indian tribes) - [Percentage of 2.a.]: **(FAR 52.219-9(d)(2)(vi))**

(b) (4) and

If your contract includes options, please include the break down here: 5 %

Base: (b) (4) and

OPT 1 (b) (4) 5 %

OPT 2 and 5 %

OPT 3 5 %

OPT 4: $ and 5 %

OPT 5: $ 5 %

OPT 6 $ and

5%

If options are applicable:
Base: 5%
OPT1: 5%
OPT 2: 5%
OPT 3: 5%
OPT 4: 5%
OPT 5: 5%
OPT 6: 5%

g. Total dollars planned to be subcontracted to women-owned small business concerns- [Percentage of 2.a.]: **(FAR 52.219-9(d)(2)(vii))**

[redacted] (b) (4)	and	5 %	5%
If your contract includes options, please include the break down here:			If options are applicable: Base: 5% OPT 1: 5% OPT 2: 5% OPT 3: 5% OPT 4: 5% OPT5: 5% OPT 6: 5%
Base: [redacted] (b) (4)			
OPT 1: [redacted] (b) (4)	and	5 %	
	and	5 %	
OPT 2:	and		
OPT 3:		5 %	
OPT 4: $	and	5 %	
OPT 5 $	and	5 %	
OPT 6 $	and		

h. Total Subcontracting Dollars & Percentage with "Other" than Small Businesses (i.e., large companies, non profits, etc.) [Percentage of 2.a.]: **(HHS OSDBU)**			67%
[redacted] (b) (4)	and	67 %	If options are applicable: Base: 67% OPT 1: 67% OPT 2: 67% OPT 3: 67% OPT 4: 67% OPT5: 67% OPT 6: 67%
If your contract includes options, please include the break down here:			
Base: [redacted] (b) (4)	and		
OPT 1: [redacted] (b) (4)		67 %	
OPT 2	and	67 %	
OPT 3	and	67 %	
OPT 4: $	and	67 %	
OPT 5 $	and	67 %	
OPT 6 $	and		

i. Subcontracting Opportunities (description of all principal products/services to be subcontracted to all types of concerns listed in 2.b.-2.f.) **(FAR 52.219-9(d)(3))**:

Provide a description of ALL the products and/or services to be subcontracted under this contract, and indicate the size and type of business supplying them (include all that apply):

Products and/or Services	Other	Small Business	SDB	WOSB	Hubz	VOSB	SDVOSB
Subcontractors	X						
Material and Supplies	X	X	X	X	X	X	X
Consultant	X						
Travel		X		X			
Other Direct Cost	X	X	X	X	X	X	X

j. Please describe the methodology used to develop goals & identify potential sources (e.g. historical trends, information on technical and competitive bidding, formula for calculating goals, etc.) (**FAR 52.219-9(d)(3)**)

An assumption was made that the full value of this contract were used to determine the proposed amounts subject to .subcontracting. Historical data, request for quotation and existing contracted Small MWBE were used.

k. Indirect costs have ☐ have not x been included in the dollar and percentage subcontracting goals above (**check one**).

If indirect costs have been included in establishing subcontracting goals, please provide a description of the method used to determine the proportionate share of indirect costs to be incurred with all types of concerns listed in 2.b.-2.f. **(FAR 52.219-9(d)(6)):**

SUBCONTRACTING PLAN REQUIREMENTS (con't)

3. Please enter the following information for the individual who will administer your Subcontracting Program: **(FAR 52.219-9(d)(7))**

Name: Audrey Kelley Title: Supplier Diversity Manager

Address: 1599 Clifton Road, Suite 3.305

Telephone: 404-727-7845 Email: akell03@emory.edu

Duties: Does the individual named above have general overall responsibility for the company's subcontracting program, i.e., developing, preparing, and executing subcontracting plans and monitoring performance relative to the requirements of those subcontracting plans and perform the following duties? xyes ☐no

Additionally, please respond whether or not the individual who will administer you subcontracting program conducts the following:

1. Developing and promoting company-wide policy initiatives that demonstrate the company's support for awarding contracts and subcontracts to SB, SDB, WOSB, HUBZone, VOSB and SDVOSB concerns; and for assuring that these concerns are included on the source lists for solicitations for products and services they are capable of providing; xyes ☐no
2. Developing and maintaining bidder source lists of SB, SDB, WOSB, HUBZone, VOSB and SDVOSB concerns from all possible sources; xyes ☐no
3. Ensuring periodic rotation of potential subcontractors on bidder's lists; x yes ☐no
4. Assuring that SB, SDB, WOSB, HUBZone, VOSB and SDVOSB businesses are included on the bidders' list for every subcontract solicitation for products and services that they are capable of providing. X yes ☐no
5. Ensuring that Requests for Proposals (RFPs) are designed to permit the maximum practicable participation of SB, SDB, WOSB, HUBZone, VOSB and SDVOSB concerns. X yes ☐no
6. Reviewing subcontract solicitations to remove statements, clauses, etc., which might tend to restrict or prohibit small, 8(a), SDB, WOSB, HUBZone, VOSB and SDVOSB small business participation. X yes ☐no
7. Accessing various sources for the identification of SB, SDB, WOSB, HUBZone, VOSB and SDVOSB concerns to include the System for Award Management (http://sam.gov), local small business and minority associations, local chambers of commerce and Federal agencies' Small Business Offices;x yes ☐no
8. Establishing and maintaining contract and subcontract award records; x yes ☐no
9. Participating in Business Opportunity Workshops, Minority Business Enterprise Seminars, Trade Fairs, Procurement Conferences, etc; x yes ☐no
10. Ensuring that SB, SDB, WOSB, HUBZone, VOSB and SDVOSB concerns are made aware of subcontracting opportunities and assisting concerns in preparing responsive bids to the company; x yes ☐no
11. Conducting or arranging for the conduct of training for purchasing personnel regarding the intent and impact of Section 8(d) of the Small Business Act, as amended; x yes ☐no
12. Monitoring the company's subcontracting program performance and making any adjustments necessary to achieve the subcontract plan goals; x yes ☐no
13. Preparing and submitting timely, required subcontract reports; x yes ☐no
14. Conducting or arranging training for purchasing personnel regarding the intent and impact of 8(d) of the Small Business Act on purchasing procedures;and x yes ☐no
15. Coordinating the company's activities during the conduct of compliance reviews by Federal agencies. X yes ☐no

(If **NO** is checked for any of the duties above, please provide who in the company performs those duties, or indicate why the duties are not performed in your company on a separate sheet of paper and submit with the proposed subcontracting plan.)

Additional duties of the individual: Manage Supplier Diversity Program at Emory University and interface with the SAM system on a daily basis, and Emory's Outreach progam.

4. Please describe your efforts to ensure that Small Businesses (incl. SDB, WOSB, HUBZone, SDVOSB) have an equitable opportunity to compete for subcontracts: **(FAR 52.219-9(d)(8))**

These efforts include, but are not limited to, the following activities:

a. Outreach efforts to obtain sources: (1) Contact minority and small business trade associations; (2) contact business development organizations and local chambers of commerce; (3) attend SB, SDB, WOSB, HUBZone, VOSB and SDVOSB procurement conferences and trade fairs; (4) review sources from the System for Award Management (http://www.sam.gov); (5) review sources from the Small Business Administration (SBA), Dynamic Small Business Search database (DSBS) http://dsbs.sba.gov/); (6) Consider using other sources such as the National Institutes of Health (NIH) e-Portals in Commerce, (e-PIC), (http://epic.od.nih.gov/)). The NIH e-PIC is not a mandatory source; however, it may be used at the offeror's discretion; and (7) Utilize newspaper and magazine ads to encourage new sources.

b. Internal efforts to guide and encourage purchasing personnel: (1) Conduct workshops, seminars and training programs; (2) Establish, maintain, and utilize SB, SDB, WOSB, HUBZone, VOSB and SDVOSB source lists, guides, and other data for soliciting subcontractors; and (3) Monitor activities to evaluate compliance with the subcontracting plan.

Efforts Described: Emory University's Procurement Department conducts Opportunity fairs, encourages the utilization of all Small MWBEs in support of good faith efforts and community support. Also, we are a member of the Georgia Minority Supplier Development Council (GMSDC). This provides networking opportunities with Small, Minority and Woman-Owned Business Enterprise (Small,MWBEs).

Emory University's Plan Administrator is a Certified Diversity Professional through the Institute for Diversity (idc).

Internal Efforts To Guide and Encourage Purchasing Personnel:

1. Conduct meeting, seminars and training on "How to work with Emory University."
2. Establish, maintain and utilize SB, SDB, WOSB, HubZone, VOSB and SDVOSB source list, guides, and other data for Soliciting subcontractors.
3. Monitor activities to evaluate compliance with the subcontracting plan.
4. Emory University's Procurement Department monitor both federal and non-federal spend with SB, WOSB, HUBZONE VOSB and SDVOSB.

Additional efforts: Provide Training on SAM system and conduct and maintain electronic BID process.

5. Flow Down Clause: **(FAR 52.219-9(d)(8))**

The contractor agrees to include the provisions under FAR 52.219-8, "Utilization of Small Business Concerns," in all acquisitions exceeding the simplified acquisition threshold that offers further subcontracting opportunities. All subcontractors, except small business concerns, that receive subcontracts in excess of $700,000 ($1,500,000 for construction) must adopt and comply with a plan similar to the plan required by FAR 52.219-9, "Small Business Subcontracting Plan." Note: In accordance with FAR 52.212-5(e) and 52.244-6(c)(2) the contractor is not required to include flow-down clause FAR 52.219.-9 if it is subcontracting commercial items.

6. Reporting and Cooperation: **(FAR 52.219-9(d)(10)); (FAR 19.704(a)(10))**

The contractor gives assurance of 1) cooperation in any studies or surveys that may be required; 2) submission of periodic reports which illustrate compliance with the subcontracting plan; 3) submission of its Individual Subcontracting Report (ISR) and Summary Subcontract Report (SSR); and 4) subcontractors submission of ISRs and SSRs. ISRs and SSRs shall be submitted via the Electronic Subcontracting Reporting System (eSRS) website https://esrs.symplicity.com/index?_tab=signin&cck=1

Please refer to FAR Part 19.7 or contact the Contracting Officer for regulatory reporting requirements and other obligations you are accepting as part of your signing of this document and acceptance of any subsequent contract award that may be granted.

7. Record keeping: **(FAR 52.219-9(d)(11))**

FAR 52.219-9(d)(11) requires a list of the types of records your company will maintain to demonstrate the procedures adopted to comply

with the requirements and goals in the subcontracting plan.

Contractor acknowledges and agrees to record keeping obligation expressed at FAR 52.219-9(d)(11). yes **x** no ☐

8. Assurances of Good Faith Effort, and the submission of explanations when failing to acquire as stated in Good Faith Effort: **(FAR 52.219-9(d)(12-13))**

Contractor hereby makes the following assurances:
(1) that contractor will make a good faith effort to acquire articles, equipment, supplies, services, or materials, or obtain the performance of construction work from the small business concerns that it used in preparing the bid or proposal, in the same or greater scope, amount, and quality used in preparing and submitting the bid or proposal yes x no ☐; and
(2) that the Contractor will provide the Contracting Officer with a written explanation if the Contractor fails to acquire articles, equipment, supplies, services or materials or obtain the performance of construction work within 30 days of contract completion and as required under FAR 19.7. yes x no ☐

9. Assurances that the Contractor will not prohibit a subcontractor from discussing with the Contracting Officer any material matter pertaining to payment to or utilization of a subcontractor: **(FAR 52.219-9(d)(14))**

Contractor hereby makes the assurance that the Contractor will not prohibit a subcontractor from discussing with the Contracting Officer any material matter pertaining to payment to or utilization of a subcontractor. yes x no ☐

10. Assurances of Timely Payments to Subcontractors: **(FAR 52.219-9(d)(15))**

FAR 19.702 requires your company to establish and use procedures to ensure the timely payment of amounts due pursuant to the terms of your subcontracts with SB concerns, SDB, WOSB, HUBZone, VOSB and SDVOSB concerns.

Your company has established and use such procedures yes x no ☐. Additionally, Contractor makes an assurance that Contractor will pay its small business subcontractors on time and in accordance with the terms and conditions of the underlying subcontract, and notify the contracting officer when the prime contractor makes either a reduced or an untimely payment to a small business subcontractor yes x no ☐.

Signature Page

1. Contractor makes the following representation: I have reviewed FAR Part 19.704 and FAR Clause 52.219-9, and this Subcontracting Plan is in compliance. yes x no ☐

This Subcontracting Plan was submitted by:

Name: Audrey Kelley Title: Supplier Diversity Manager

Signature: (b) (6)

Address: 1599 Clifton Road, Suite 3.305Atlanta, Georgia 3322

Telephone: 404-727-7845 Email: akell03@emory.edu

Date: July 26, 2019

HHS SUBCONTRACTING PLAN TEMPLATE

*A Subcontracting Plan is required if the estimated cost of the contract **may exceed $700,000 ($1,500,000 for construction)** Small businesses are excluded.*

The following outline meets the minimum requirements of section 8(d) of the Small Business Act, as amended, and implemented by the Federal Acquisition Regulations (FAR) Subpart 19.7. The U.S. Department of Health and Human Services (HHS), Office of Small and Disadvantaged Business Utilization (OSDBU) recommends that offerors use the following format to submit proposed Individual Subcontracting Plans. It is not intended to replace any existing Corporate/Commercial Plan that is more extensive.

Questions should be forwarded to the Contracting Officer and/or Small Business Subcontracting Program Manager.

Safety and Health, HHSAR 352.223-70 (DEC 2015)

a. To help ensure the protection of the life and health of all persons, and to help prevent damage to property, the Contractor shall comply with all Federal, State, and local laws and regulations applicable to the work being performed under this contract. These laws are implemented or enforced by the Environmental Protection Agency, Occupational Safety and Health Administration (OSHA) and other regulatory/enforcement agencies at the Federal, State, and local levels.
    1. In addition, the Contractor shall comply with the following regulations when developing and implementing health and safety operating procedures and practices for both personnel and facilities involving the use or handling of hazardous materials and the conduct of research, development, or test projects:
        I. 29 CFR 1910.1030, Bloodborne pathogens; 29 CFR 1910.1450, Occupational exposure to hazardous chemicals in laboratories; and other applicable occupational health and safety standards issued by OSHA and included in 29 CFR part 1910. These regulations are available at https://www.osha.gov/.
        II. Nuclear Regulatory Commission Standards and Regulations, pursuant to the Energy Reorganization Act of 1974 (42 U.S.C. 5801 et seq.). The Contractor may obtain copies from the U.S. Nuclear Regulatory Commission, Washington, DC 20555-0001.

    2. The following Government guidelines are recommended for developing and implementing health and safety operating procedures and practices for both personnel and facilities:
        I. Biosafety in Microbiological and Biomedical Laboratories, CDC. This publication is available at http://www.cdc.gov/biosafety/publications/index.htm.
        II. Prudent Practices for Safety in Laboratories (1995), National Research Council, National Academy Press, 500 Fifth Street NW., Lockbox 285, Washington, DC 20055 (ISBN 0-309-05229-7). This publication is available at http://www.nap.edu/catalog/4911/prudent-practices-in-the-laboratory-handling-and-disposal-of-chemicals.

b. Further, the Contractor shall take or cause to be taken additional safety measures as the Contracting Officer, in conjunction with the Contracting Officer's Representative or other appropriate officials, determines to be reasonably necessary. If compliance with these additional safety measures results in an increase or decrease in the cost or time required for performance of any part of work under this contract, the Contracting Officer will make an equitable adjustment in accordance with the applicable ``Changes'' clause set forth in this contract.

c. The Contractor shall maintain an accurate record of, and promptly report to the Contracting Officer, all accidents or incidents resulting in the exposure of persons to

toxic substances, hazardous materials or hazardous operations; the injury or death of any person; or damage to property incidental to work performed under the contract resulting from toxic or hazardous materials and resulting in any or all violations for which the Contractor has been cited by any Federal, State or local regulatory/enforcement agency. The report citing all accidents or incidents resulting in the exposure of persons to toxic substances, hazardous materials or hazardous operations; the injury or death of any person; or damage to property incidental to work performed under the contract resulting from toxic or hazardous materials and resulting in any or all violations for which the Contractor has been cited shall include a copy of the notice of violation and the findings of any inquiry or inspection, and an analysis addressing the impact these violations may have on the work remaining to be performed. The report shall also state the required action(s), if any, to be taken to correct any violation(s) noted by the Federal, State, or local regulatory/enforcement agency and the time frame allowed by the agency to accomplish the necessary corrective action.

d. If the Contractor fails or refuses to comply with the Federal, State or local regulatory/enforcement agency's directive(s) regarding any violation(s) and prescribed corrective action(s), the Contracting Officer may issue an order stopping all or part of the work until satisfactory corrective action (as approved by the Federal, State, or local regulatory/enforcement agencies) has been taken and documented to the Contracting Officer. No part of the time lost due to any such stop work order shall form the basis for a request for extension or costs or damages by the Contractor.

e. The Contractor shall insert the substance of this clause in each subcontract involving toxic substances, hazardous materials, or hazardous operations. The Contractor is responsible for the compliance of its subcontractors with the provisions of this clause.

(End of clause)

DISCLOSURE OF LOBBYING ACTIVITIES

Approved by OMB
0348-0046

Complete this form to disclose lobbying activities pursuant to 31 U.S.C. 1352
(See reverse for public burden disclosure.)

1. Type of Federal Action:	2. Status of Federal Action:	3. Report Type:
☐ a. contract b. grant c. cooperative agreement d. loan e. loan guarantee f. loan insurance	☐ a. bid/offer/application b. initial award c. post-award	☐ a. initial filing b. material change **For Material Change Only:** year _____ quarter _____ date of last report _____

4. Name and Address of Reporting Entity:	5. If Reporting Entity in No. 4 is a Subawardee, Enter Name and Address of Prime:
☐ Prime ☐ Subawardee Tier _____, *if known*: **Congressional District**, *if known*: 4c	**Congressional District**, *if known*:
6. Federal Department/Agency:	**7. Federal Program Name/Description:** CFDA Number, *if applicable*: _____
8. Federal Action Number, *if known*:	**9. Award Amount**, *if known*: $
10. a. Name and Address of Lobbying Registrant (*if individual, last name, first name, MI*):	**b. Individuals Performing Services** (*including address if different from No. 10a*) (*last name, first name, MI*):
11. Information requested through this form is authorized by title 31 U.S.C. section 1352. This disclosure of lobbying activities is a material representation of fact upon which reliance was placed by the tier above when this transaction was made or entered into. This disclosure is required pursuant to 31 U.S.C. 1352. This information will be available for public inspection. Any person who fails to file the required disclosure shall be subject to a civil penalty of not less than $10,000 and not more than $100,000 for each such failure.	Signature: _____ Print Name: _____ Title: _____ Telephone No.: _____ Date: _____
Federal Use Only:	Authorized for Local Reproduction Standard Form LLL (Rev. 7-97)

PRINT

INSTRUCTIONS FOR COMPLETION OF SF-LLL, DISCLOSURE OF LOBBYING ACTIVITIES

This disclosure form shall be completed by the reporting entity, whether subawardee or prime Federal recipient, at the initiation or receipt of a covered Federal action, or a material change to a previous filing, pursuant to title 31 U.S.C. section 1352. The filing of a form is required for each payment or agreement to make payment to any lobbying entity for influencing or attempting to influence an officer or employee of any agency, a Member of Congress, an officer or employee of Congress, or an employeeof a Member of Congress in connection with a covered Federal action. Complete all items that apply for both the initial filing and material change report. Refer to the implementing guidance published by the Office of Management and Budget for additional information.

1. Identify the type of covered Federal action for which lobbying activity is and/or has been secured to influence the outcome of a covered Federal action.

2. Identify the status of the covered Federal action.

3. Identify the appropriate classification of this report. If this is a followup report caused by a material change to the information previously reported, enter the year and quarter in which the change occurred. Enter the date of the last previously submitted report by this reporting entity for this covered Federal action.

4. Enter the full name, address, city, State and zip code of the reporting entity. Include Congressional District, if known. Check the appropriate classification of the reporting entity that designates if it is, or expects to be, a prime or subaward recipient. Identify the tier of the subawardee, e.g., the first subawardee of the prime is the 1st tier. Subawards include but are not limited to subcontracts, subgrants and contract awards under grants.

5. If the organization filing the report in item 4 checks "Subawardee," then enter the full name, address, city, State and zip code of the prime Federal recipient. Include Congressional District, if known.

6. Enter the name of the Federal agency making the award or loan commitment. Include at least one organizationallevel below agency name, if known. For example, Department of Transportation, United States Coast Guard.

7. Enter the Federal program name or description for the covered Federal action (item 1). If known, enter the full Catalog of Federal Domestic Assistance (CFDA) number for grants, cooperative agreements, loans, and loan commitments.

8. Enter the most appropriate Federal identifying number available for the Federal action identified in item 1 (e.g., Request for Proposal (RFP) number; Invitation for Bid (IFB) number; grant announcement number; the contract, grant, or loan award number; the application/proposal control number assigned by the Federal agency). Include prefixes, e.g., "RFP-DE-90-001."

9. For a covered Federal action where there has been an award or loan commitment by the Federal agency, enter the Federal amount of the award/loan commitment for the prime entity identified in item 4 or 5.

10. (a) Enter the full name, address, city, State and zip code of the lobbying registrant under the Lobbying Disclosure Act of 1995 engaged by the reporting entity identified in item 4 to influence the covered Federal action.

    (b) Enter the full names of the individual(s) performing services, and include full address if different from 10 (a). Enter Last Name, First Name, and Middle Initial (MI).

11. The certifying official shall sign and date the form, print his/her name, title, and telephone number.

According to the Paperwork Reduction Act, as amended, no persons are required to respond to a collection of information unless it displays a valid OMB Control Number. The valid OMB control number for this information collection is OMB No. 0348-0046. Public reporting burden for this collection of information is estimated to average 10 minutes per response, including time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding the burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to the Office of Management and Budget, Paperwork Reduction Project (0348-0046), Washington, DC 20503.